GENETICS

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ABSTRACTS OF PAPERS PRESENTED AT THE 1957 MEETINGS OF THE GENETICS SOCIETY OF AMERICA

STANFORD, CALIFORNIA, AUGUST 26 TO 28, 1957

ALLEN, SALLY LYMAN, and IRENE V. SCHENSTED, University of Michigan, Ann Arbor, Mich.: *Subnuclear segregation in Tetrahymena pyriformis.*—As a rule, macronuclear differentiation and separation results in the establishment of one of five mating types in cells of Family A, variety 1, of *T. pyriformis*. However, the establishment of dual systems in "selfers" is the most common exception to this rule. From selfers stable cells of two different pure types may be generated. When the kinetics of stabilization of two qualitatively distinct systems (I-VI and V-VI) were examined, similar properties emerged: (1) Stabilization of only one of the two mating types occurs in a given subclone at a given time; (2) The total rate of stabilization reaches an early equilibrium value; (3) The rates of stabilization to the two types are inversely correlated in time and approach equality. These results could be interpreted as due to the segregation of subnuclear elements from the macronucleus, where the total number of subnuclei (N) is estimated as 45. A selfer would contain a mixture of two types of subnuclei and stabilization of a pure mating type would be observed following the elimination of all but one type of subnucleus. Theoretical curves for various proportions of subnuclei (where N = 45) have been derived and applied to the data. The agreement between the theoretical and experimental curves is excellent and suggests that subnuclear segregation may in fact be the mechanism whereby the stabilization of pure mating types is effected from a selfer, although alternative mechanisms have not been eliminated. (Work supported by grants to D. L. NANNEY from the National Science Foundation and to C. LEVINTHAL from the U. S. Public Health Service).

ALEXANDER, MARY L., Biology Department, University of Texas, M. D. Anderson Hospital, Houston, Texas: *Dominant lethal damage in the immature germ cells of Drosophila virilis from 200 Kv X-ray, 1.17–1.33 Mev gamma rays and 22 Mv X-ray.*—Young males of *D. virilis* (18–21 hours old) were treated with one of three types of irradiation: 200 Kv X-ray, 1.17–1.33 Mev gamma rays from a cobalt-60 source, and 22 Mv X-ray from a betatron source. The treated males were remated every two days to females which were obtained from a heterosis cross of two other *virilis* strains. Samples of the germ cells treated in various pre- and postmeiotic stages were obtained by this remating procedure. The dominant lethal damage in the different types of cells was measured by the percentage of pupae development from the egg samples obtained from females used for the different mating periods. Translocation tests were used to distinguish the postmeiotic from meiotic and premeiotic cells.—With all three types of radiations, the highest percentage of dominant lethals in the postmeiotic cells was produced in the young spermatids (mating period E). Less damage was observed in the more mature cells. With the dose expressed in rads, the gamma rays and 22 Mv X-rays were similar in their efficiency for producing dominant lethal damage and with 200 Kv X-ray as the standard radiation, both gave an average relative biological efficiency of 0.72 for postmeiotic cells.—The high proportion of dominant lethals induced in young spermatids was retained in sperm samples from germ cells treated in the meiotic and premeiotic stages both with gamma rays and 22 Mv X-rays. With 200 Kv X-rays, however, dominant lethal damage decreased more rapidly through these mating periods. The characteristics of the radiation damage in both the pre- and postmeiotic cells for each of the three types of radiation were consistent at levels of 500, 1000 and 2000r.

ALTENBURG, EDGAR, and LUOLIN S. ALTENBERG, Rice Institute, Houston, Texas: *The mutagenicity of disuccinoyl monoperoxide in Drosophila.*—When disuccinoyl monoperoxide is dissolved in water, it forms succinic peracid, an active peroxidizing agent. A 1% solution of disuccinoyl monoperoxide, when placed in contact for 20 minutes with the dechorionated polar caps of Drosophila eggs (containing the cells of the early germ track), induced a recessive lethal mutation rate of 1.5 ± 0.7% in the second pair of autosomes. The lethals were detected by means of MULLER's "sifter" technique,

35 males being tested. A 0.1% concentration of disuccinoyl monoperoxide failed to induce a mutation rate in this material. Inactivation and mutagenesis in microorganisms caused by this peroxide have been reported by CHEVALIER and LATARJET. (This work was supported by a grant received from the American Cancer Society.)

ALTENBURG, EDGAR, and LUOLIN S. ALTENBURG, Rice Institute, Houston, Texas: *The nonlinear increase in frequency of lethal mutations induced by low doses of ultraviolet in Drosophila.*—Drosophila eggs at the polar cap stage were dechorionated and irradiated with doses of 2537Å ultraviolet from a germicidal lamp at 180 cm for ¾, 1-½, 2, 2-½, 3, 3-½, 4, 7, and 10 minutes (the ultraviolet doses ranging from 40 to 500 ergs/mm²). The males that hatched were tested for the production of recessive lethal mutations in the second pair of autosomes by the "sifter" technique of MULLER. The induced mutation rates obtained after these treatments were 1.1 ± 0.2%, 4.7 ± 1.0, 4.1 ± 1.7 6.0 ± 1.8, 5.9 ± 1.9, 5.1 ± 1.5, 5.7 ± 2.2, and 2.9 ± 1.3, respectively. The increase in mutation rate from 1.1 ± 0.2% at the lowest dose to 4.7 ± 1.0% at twice that dose is sufficiently large to indicate that the rate may rise as the square of the dose, or at least at a much greater than linear rate. The ratio of the mutation rate at the lowest dose and that at twice the dose is 4.3 ± 1.2%, which indicates that there is less than a 3% chance that the ratio of the mutation rate at the higher dose to that at the lower is 2:1.—Further increase in dose failed to produce a significant increase in the mutation rate until a dose thirteen times that of the lower dose was reached (10 minutes at 180 cm), at which dose the rate dropped to 2.9 ± 1.3%. These results confirm those reported previously (1952) at a different intensity but with a comparable dosage range. (This work has been supported by a grant received for work of EDGAR ALTENBURG and associates from the National Institutes of Health, United States Public Health Service.)

ALTENBURG, LUOLIN S., and EDGAR ALTENBURG, Rice Institute, Houston, Texas: *A comparison of the effect of photoreactivating light on the mutation rates produced by different doses of 2537 Å ultraviolet light.*—Photoreactivating light (3000–4200Å) when applied for 3-½ minutes immediately following treatment of the polar caps of dechorionated Drosophila eggs for 3-½ minutes at 180 cm (180 ergs/mm², a medium dose in our experiments), lowered the induced recessive lethal mutation rate in the second pair of autosomes from 5.7 ± 1.9% to 0.8 ± 0.4%. When the posttreatment by photoreactivating light was continued for 10 minutes, there was no significant difference between the treated and control rates, indicating that the induced mutation rate was completely obliterated. When photoreactivating light was applied simultaneously with the 3-½-minute treatment with 2537Å ultraviolet, an induced rate of 4.9 ± 1.7% was obtained, indicating that no appreciable amount of photorepair took place at this dose under these conditions.—When a dose of 2537Å ultraviolet was applied for 10 minutes at 180 cm (516 ergs/mm²), a 2.9 ± 1.3% mutation rate was induced. By means of a 10-minute posttreatment with photoreactivating light, this rate was increased to 10.0 ± 2.0%, an effect of the photoreactivating light contrary to the marked lowering of the rate when a moderate dose of 2537Å ultraviolet was used. MEYER on the basis of unpublished data (1954) has suggested the possibility of a similar "reverse relation" in Drosophila. (This work has been supported by a grant received for work of EDGAR ALTENBURG and associates from the National Institutes of Health, United States Public Health Service.)

ALTENBURG, LUOLIN S., and EDGAR ALTENBURG, Rice Institute, Houston, Texas: *The mutagenicity of 4-dimethylaminoazobenzene (butter yellow) in Drosophila.*—Drosophila eggs, deposited on ordinary food medium containing 1% by weight of the carcinogenic agent 4-dimethylaminoazobenzene (butter yellow), were allowed to develop into adults and the males tested by MULLER's "sifter" technique for recessive lethals in the second pair of autosomes. A low but significant induced mutation rate of 1.2 ± 0.5% from 70 males so tested was found.—When Drosophila eggs were treated for 20 minutes at the polar cap stage in development by immersing the polar cap ends of the eggs in a 2% aqueous solution of the carcinogen, a mutation rate not significantly different from the spontaneous rate was obtained from 62 males. Since butter yellow absorbs ultraviolet light and so might possibly become more reactive in combination with it, a combined treatment of 20 minutes of a 2% aqueous solution

of the carcinogen, followed by 45 seconds of 2537Å ultraviolet at 180 cm, was administered to the polar caps of Drosophila eggs. This treatment likewise failed to produce a mutation rate in 51 tested ♂♂ that was significantly different from that of the ultraviolet alone. It appears therefore that it is necessary for 4-dimethylaminoazobenzene to enter into the metabolic cycle of this organism in order to exert its mutagenic effectiveness and that it may act by the production of some intermediate substance. This has a parallel in the observation that it is effective in producing liver tumors in rats only when it is ingested with the food but not when locally applied. (This work was supported by a grant received from the American Cancer Society.)

BAILEY, D. W., Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine: *Spontaneous genetic differentiation of sublines of inbred mouse strains.*—The rates of differentiation of sublines of two inbred strains (BALB/c and C57Bl/6) of the house mouse were estimated using as criteria the variances of the subline means of six skeletal measurements (ulna, ilium and skull dimensions). Papain-prepared skeletons of adult females were used. Thirty-four mice represented each of the BALB/c sublines, whereas 54 mice represented each of the C57Bl/6 sublines. The values were computed in units of the genetic variance (derived from independent data) of a theoretical population of fully inbred strains randomly derived from a panmictic population. It was estimated that the means of two BALB/c sublines (separated for 10 generations) had diverged, as an average for the six traits, at the rate of 0.0010 units/generation. The two C57Bl/6 sublines (separated for 53 generations) had diverged at the rate of 0.0003 units/generation. The range of the estimates for individual traits was from 0 to 0.0022 units/generation.

BAKER, W. K., and JANICE B. SPOFFORD, University of Chicago, Chicago, Ill.: *A chromatographic analysis of white-variegation in Drosophila melanogaster.*—This study concerns how the different Y chromosome fragments modify the expression of the w^+ locus transposed into centromeric heterochromatin. Heads of individual flies were removed, the pigmented area estimated visually and then each head squashed on paper, developed in a butanol solvent and examined under ultraviolet light. The fluorescent substances were eluted from two spots with distinctive Rf's and measured in a photofluorometer. All genotypes compared were co-isogenic except for the Y fragment, the variable studied.—Variegated eyes yield a unique Rf spot which fluoresces green, absent from wild type and white eyes. Two way chromatograms indicate that this spot probably contains a single substance. The various Y fragments have different potencies in suppressing variegation. For example, in eyes of attached-X transposition heterozygotes, Y^{cL} and the common Y^S produce larger pigmented areas than does the intact Y; the latter, more than sc^{v1} Y^S or a Y^S: y^+ bb^+ fragment from sc^8·Y: *bw*. The amount of fluorescence in both spots from each head is correlated positively with the size of the pigmented area—the yellow fluorescence of the low Rf spot linearly but loosely, the blue and green fluorescence of the high Rf spot curvilinearly and closely for areas under 50%. Y^{cL} and Y^S engender somewhat more fluorescence in both spots than the Y and considerably more than sc^{v1} Y^S or Y^S: y^+ bb^+ do. In these respects, Y^{cL} is equivalent to Y^S; $sc^{v1}Y^S$, to Y^S: y^+ bb^+.—These studies may disclose differentiation of the Y chromosome into functional units which determine the expression of position effect.

BECKER, W. A. University of California, Berkeley and Western Washington Experiment Station, Puyallup, Wash.: *Effect of the environment upon the uncontrolled phenotypic variability of rat body weight.*—The hypothesis that the uncontrolled phenotypic variability of rat body weight is reduced when an environmental factor is changed to a condition favorable for general development was tested by a series of experiments. All data were transformed to log grams to minimize the undesirable correlation between the mean and the standard deviation. Reciprocal backcrosses indicated that the animals grown under the better maternal environment of the F_1 dams were less variable than those rats littered and raised by the inbred dams. Starvation for one day of 98-day old rats reduced the mean and raised the variability. When the rats were placed back on their normal diet the variability dropped in both males and females. Rats of an inbred strain were intermittently starved for three days a week from weaning to 98 days of age. Backcross animals were also starved for two days a week. Compari-

sons were made between the within-litter mean squares of the starved rats and full fed controls. The data, in general, supported the hypothesis. A tentative biological principle is suggested that the slower the growth the greater the variability within a group of organisms.

BELL, A. E., C. H. MOORE, and D. C. WARREN, Purdue University, Lafayette, Indiana: *Genetic changes in closed populations of Drosophila melanogaster under continuous selection.*—In the first of two selection experiments comparing individual and family selection within a closed population with three other methods of selection for the improvement of quantitative traits, selection was based on a performance index combining fecundity and egg size. A peak in performance was observed in the closed population at generation 5 even though selection was continued for another 11 generations and continued improvement occurred under the other methods. In the second experiment covering a period of 40 generations, selection was based solely on fecundity and the closed population (BROWN and BELL 1957) showed that neither lethal, sterility nor subvital factors were responsible for the failure to respond to selection.—Statistical analyses revealed that phenotypic variation within the closed populations remained relatively unchanged throughout both experiments with selection differentials of about one standard deviation per generation. A negative genetic correlation (−.10) between the components of the selection index in Experiment 1 plus a decline in the additive genetic variation for fecundity are concluded to be the major factors contributing to the observed plateau in performance. In the second experiment, an exhaustion of the additive genetic variation as estimated by the regression of progeny performance on that of their dam's can solely account for the lack of response to selection beyond the 7th generation.—Heritability estimates (intraclass correlations) which include a portion of the nonadditive genetic variation, remained large enough throughout both experiments to suggest that considerable dominance or epistatic variation remained in both closed populations. (Supported in part by grants from the Rockefeller Foundation and the National Science Foundation.)

BENDER, M. A., The Johns Hopkins University, Baltimore, Maryland: *X-ray induced chromosome aberrations in normal diploid human tissue cultures.*—A strain of diploid (e.g., n = 23) epithelial cells was isolated from a normal human kidney which was obtained surgically. Material from the first and second passages was grown on cover slips in Leighton tubes. Two groups of cultures were X-irradiated, one group receiving about 25r and the other about 50r. A third group of cultures served as a control. At various intervals after irradiation, cultures from all three groups were fixed and stained by the Feulgen method.—It was found that mitosis ceased after irradiation and did not occur again until 36 hours after irradiation. Mitoses were abundant in material fixed 40, 50 and 72 hours after irradiation. No chromosome aberrations of any kind were found in the unirradiated control material. Chromosome aberrations of several types were found in both groups of irradiated material. These aberrations included chromatid and isochromatid deletions and exchanges.—Methods for measuring the frequency of the various types of aberrations are being developed. The results to date indicate that it is possible to use diploid human material in tissue culture for the quantitative study of chromosome damage produced by ionizing radiations. (These studies were carried out under United States Atomic Energy Commission Contract AT(30-1), 1939, and aided by a Research Fellowship from the National Institutes of Health, United States Public Health Service.)

BIANCHI, A., Centro di Genetica del C. N. R., Pavia, Italy: *Gametophyte factors in maize-teosinte derivatives.*—The maize kernal, and especially its highly evolved endosperm, is a structure where mutants factors are liable to prompt detection with relatively high frequency. But when appropriate genetic tests are performed the maize gametophyte turns out to be, as expected, the first place in which mutant factors may show their presence. The most of the many mutants, which have been detected in maize-teosinte derivatives (Bianchi 1956, Genetics 41: 634), were recognized as defective endosperm factors. However such derivatives crossed to multiple testers showed that several *Ga* factors should be postulated to account for typical aberrant segregation data.—A male gametophyte factor has been found on the short arm of chromosome 9. Linkage with waxy factor must be very strong (*wx wx* percent 1,17% instead of 25). All the available data indicate that the *Ga* factor detected

in the maize-teosinte derivative is the same or an allele of Ga_s, described by SCHWARTZ (*Maize News Letter*, 25: 30).—Another *Ga* factor on chromosome 9 has been found, which, however gives much minor deviations of the *wx* class. The available data suggest that, if actually a *Ga* factor is involved, it should be a very weak allele of Ga_s or, as it seems more likely, a *Ga* factor loosely linked to *wx*.—A *Ga* factor should be postulated in the family 55-488, too, where the *su* class ranges from 28.5 to 35.1%; and in the family 56-392, in which the *su* percent is 21.3 (17.5 to 24.3). In both these cases progeny tests are not yet available.—A *Ga* factor, that has been lost, should be present on chromosome 7: the percentage of gl_1 was as low as 7.2% instead of the expected 25.

BLUMEL, JOHANNA, Department of Surgery, Orthopedic Division, University of Texas, Medical Branch, Galveston, Texas: *Variants of the Laurence-Moon-Bardet-Biedl syndrome and their genetic implications.*—From the genetic point of view the Laurence-Moon-Bardet-Biedl syndrome is not easily explained. The complete and classical picture includes the following six cardinal features: obesity, mental deficiency, genital dystrophy, polydactylism, retinitis pigmentosa and familial occurrence. In addition, concomitant anomalies listed by various authors include deafness, atresia ani, nystagmus, microphthalmus, strabismus, myopia, cataracts, congenital heart disease, genu valgum, hypospadias, hydronephrosis, disseminated sclerosis, hypothyroidism, diabetes insipidus, decreased carbohydrate tolerance, extra ribs, uterus duplex, coloboma of iris, et cetera. The classical syndrome is relatively rate. However, cases with one or more of the cardinal symptoms missing and cases substituting one or more of the concomitant features make up the largest group of reported cases.—Thus, when subjecting the available data to genetic analysis to test the postulated types of inheritance, one is confronted with the problem of what actually constitutes the syndrome. Any one of the cardinal features, without generalized association, has a definite pattern of inheritance, i.e. retinitis pigmentosa alone is inherited in four different ways: sex-linked recessive, sex-linked dominant, autosomal dominant and autosomal recessive. The frequent occurrence of one or several components of this condition in relatives of individuals showing the full syndrome impose additional problems. In this investigation all available cases reported in the literature and four additional cases of our own were evaluated and analysed with special reference to manifestations of human hereditary abnormalities in collaterals and ascendants. (This investigation has been supported in part by Grant No. A-848 (C) from the U. S. Public Health Service).

BRAVER, G., University of California, Berkeley, Calif.: *Crossing over in In(l)rst^3.*—*In(l)rst^3* (GRUNEBERG) is an almost entire inversion of the X chromosome of *D. melanogaster*. With break points near *rst* (1.7), and in the basal heterochromatin, the chromosome has a small noninverted euchromatic region at the tip. This distal euchromatin (essentially the *y-w* region) is followed by the inverted portion of the chromosome, so that the displaced heterochromatin lies just proximal to the white locus. GRUNEBERG and MATHER both reported an excess of crossing over in the *y-car* region of homozygous *In(l)rst^3*, which the latter attributed, in part, to exchange in the heterochromatin.—Using *In(l)rst^{3}w*, produced recently by NOVITSKI by irradiating *In(l)rst^3*, it is possible to test crossing over in the distal euchromatin. It has been found that crossing over in the *y*(0.0)-*w*(1.5) region is high, with percentages, in three matings, of 5.5 (N = 4624), 6.6 (N = 2165) and 4.4 (N = 6283). Crossover percentages for the *w-car* region were 14.6 (N = 2425) and 10.8 (N = 1093) in two tests. Values for this region are high, when compared with the 5% reported by MATHER for the comparable *y-car* region in homozygous *In(l)sc^8*.—Preliminary tests with females of the constitution *In(l)rst^3 y f/In(l)rst^3 y ac sc pn f* gave 0.57% crossing over (N= 9330) for the *ac*(0.0)-*pn*(0.8) region (including one crossover between *ac* and *sc*). Apparently, then, the increased exchange in the distal euchromatin of homozygous *In(l)rst^3* is strongly localized in the proximal half of the *y-w* region.

BREWBAKER, J. L., Brookhaven National Laboratory, Upton, N. Y.: *Pollen anatomy and self-incompatibility in plants.*—Self-incompatibility in homomorphic flowering plants has been attributed in all 30 species studied to the action of multiple alleles of a single "S" locus (rarely, of duplicate loci). Two different systems are constructed on this basic scheme: (1) the *gametophytic system* ("Nicotiana" system) in which pollen phenotype is determined autonomously by the S allele in the pollen,

and (2) the *sporophytic system* ("Composite" system) in which pollen behavior is dictated sporophytically by the maternal S genotype.—These systems differ basically only in the *time of S allele action* in microsporogenesis. This action must occur during or after meiosis in gametophytic species and premeiotically in sporophytic species.—While compiling a card file of self-incompatible species, it has become evident that all gametophytic species represent plant families having binucleate pollen grains (in which sperm formation occurs after pollen germination) while sporophytic species (with one possible exception) have trinucleate grains. The incompatibility is expressed as pollen-tube inhibition in the former (e.g. Leguminoseae, Scrophulariaceae, Solanaceae) and as the inhibition of pollen germination in the latter (e.g., Compositae, Cruciferae, Gramineae). Further, the bi- and trinucleate conditions are correlated with external pollen morphology (3- and 6-colpate respectively) in certain families (e.g., Labiatae).—The timing is relatively the same for each of these phenomena—early gene action in sporophytic, late in gametophytic species. The implications of this difference in timing for studies of inhibition in vitro and the predictive value of the correlation of pollen anatomy and incompatibility system will be discussed. (Research carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.)

BRINK, R. ALEXANDER, University of Wisconsin, Madison, Wisconsin: *The absence of a cytoplasmic factor in the genetic change in R expression in maize plants heterozygous for stippled.*—*R* (self-colored aleurone) in maize regularly changes to *R'*, a weakly pigmenting form, in *R R*st heterozygotes. The *R'* form is maintained in heterozygotes with *r* (colorless aleurone) but reverts partially (not completely as implied in Genetics 41: 872-889) toward the standard *R* level of pigmentation in *R'R'* homozygotes derived from *R R*st by selfing. Matings of the type *rr* ♀ × standard *RR*♂, in which the *rr* parent was derived from *R*st*r* by selfing, give the same darkly pigmented aleurone phenotype as results from standard *rr* ♀ × standard *RR*♂ crosses. Also, normal *R* action is not altered in *Rr* sporophytes carrying an *r* allele derived from *R*st*r* plants. This evidence points to two conclusions (1) the genetic change from *R* to *R'* is not attributable to a cytoplasmic factor and (2) the capacity of *R*st to induce the change from *R* to *R'* in *R R*st plants is not conferred upon *r* in *R*st*r* heterozygotes.

BROWN, META S., Texas Agricultural Experiment Station, College Station, Texas: *The division of univalents in Gossypium.*—Univalent chromosomes are commonly observed in many species hybrids of Gossypium and in asynaptic lines of species and hybrids. Univalents rarely divide in the first meiotic division. They are distributed at random to the poles at anaphase and divide regularly at the second division. Plants with centric fragments, which pair to form heteromorphic bivalents at metaphase I, have been recovered from monosomic lines. The recovery of these centric fragments is attributed to aberrant division of univalents. Monosomics thus possess a mechanism for restoring the 2*n* centromere number. The recovery of centric fragments provides a further mechanism whereby minor changes in chromosome complement can take place, either by crossing over with the normal homologue, or by providing a point of attachment for chance fragments from other chromosomes.

BROWN, SPENCER W., University of California, Berkeley, Calif.: *Chromosome behavior in Comstockiella sabalis* (*Comstk.*) (*Coccoidea-Diaspididae*).—Although recognized as morphologically anomalous, *C. sabalis* has been classified as a diaspidine scale. Its genetic system is unlike that of other scales studied, which are haplo-diploids, and seems to be a modification of the mealy bug (lecanoid) system in which both sexes are diploid but one haploid set in the males is heteropycnotic from early embryogeny and eventually eliminated at spermatogenesis. In *c. sabalis*, 2n = 10, one set of 5 chromosomes becomes heteropycnotic in male embryos. During spermatogenesis, the pycnotic differential disappears during prophase. Four 'bivalents' are formed at late prophase, after the chromsomes have contracted. The remaining (d) pair shows differential behavior: one chromosome is lightly stained; its homologue is heavily stained and visibly split. The components of the four bivalents seem to separate regularly at anaphase and there is no second division. Of the d-pair, the lightly stained member apparently divides regularly while its darker homologue shows frequent lagging and failure of separation. Since many anaphase figures do not show laggards factors other than simple lagging must be responsible for the complete exclusion of the d-chromosome from the two spermatid nuclei.

The excluded d-chromosomes are visible in the cytoplasm during sperm formation as two heavily stained, visibly split structures. Thus the genetic system of *C. sabalis* seems to be a reversion from the lecanoid system toward a more usual diploidy in that sperm are not formed from a single haploid set; furthermore, the behavior of the d-pair is suggestive of a sex chromosome differential.

BROWN, WM. P. and A. E. BELL (introduced by J. HOLMES MARTIN), Purdue University, Lafayette, Indiana: *Genetic analysis of a closed population of D. melanogaster which exhibited a plateau for fecundity.*—A closed population of *D. melanogaster* which had been selected continually for high fecundity for 47 generations and which continued to give significant selection differentials and heritability estimates exhibited a plateau after the seventh generation. To account for this plateau, investigations were made concerning the presence of lethal factors, sterility factors and differential viability by extracting 126 genomes for the three major chromosomes from the population through the dominant marker inversion technique.—A frequency of 5.26 percent was found for sex-linked lethals. Only three of the genomes gave evidence for autosomal lethals which is a frequency of 2.4 percent, and these were confined to chromosome II. Reproduction of the 123 remaining genome samples was nearly 100 percent which indicated that sterility factors were at a minimum. None of the preceding factors appear to have effected the plateau.—A 2^4 factorial analysis involving the factors of sex, and chromosomes I, II and III was performed to study the relative viability of different genotypes which were products of the genome isolation technique. All of the main effects except sex were significant, and of all the first and second order interactions, only sex $\times$ I and II $\times$ III were significant. In every case of significance homozygotes were superior to heterozygous inversion types. —In a subsequent analysis involving a random sample of three genomes from the closed population in all possible combinations, no significant differences were found between the homozygous and heterozygous combinations for relative viability. This finding makes possible the tentative conclusion that the genomes were very similar, or the closed population was homozygous for the factors controlling viability. (Supported in part by grants from the Rockefeller Foundation and the National Science Foundation.)

CARLSON, E. A., Indiana University, Bloomington, Ind.: *A further analysis of allelism in the dumpy series of D. melanogaster.*—Mutants of the dumpy series may be characterized by oblique wings (*o*) thoracic vortices (*v*), and a homozygous lethal effect (*l*). Heterozygous females of composition *o/lv*, *ol/v*, and *o/v* yield reciprocal recombinant types with normal distribution of the outside markers, echinoid and clot, when *o* or *ol* are taken as left of *v* or *lv*. Linkage values are sensibly alike for *o* and *ol*, but *v* gives more crossing over with *o* than does *lv* (structural change?). Three genotypes manifest the *olv* phenotype: the spontaneous mutant dp^T; the recombinant arising from *o/lv*; and the recombinant arising from *ol/v*. No crossovers were obtained from $dp^T/^+$ females. This indicates that dp^T represents a gene-mutation or a line-mutation (e.g. deletion, inversion), not a multipoint one. Analysis of the spontaneous mutant *dp* (phenotypically similar to the recombinant arising from *o/v*) similarly shows a unitary (gene- or line-mutation) origin of *dp*. Multiple-effect mutants of the dumpy series are induced far oftener than single-effect ones. Failure to break down any recombinant *olv* into other alleles than those that produced it indicates nonseparability of *l* from the *o* or *v*, respectively. At the same time, the interaction in the compound *ol/lv* that produces the lethal effect supports a pseudoallelic interpretation. This is further upheld by discernible differences between cis and trans configurations of *o* with *lv* and *ol* with *v*. Functional relations between the *o* and *v* regions are also demonstrated by use of modifiers and other techniques. (This investigation was supported by a predoctoral fellowship from the National Institutes of Health, Public Health Service and by a grant for work of H. J. MULLER and associates from the U. S. Atomic Energy Commission (Contract AT (11-1)-195).)

CARSON, HAMPTON L. Department of Zoology, Washington University, St. Louis 5, Missouri: *Production of biomass as a measure of fitness of experimental populations of Drosophila.*—Each population is maintained in a chamber made of an ordinary shell vial (9.5 $\times$ 2.5 cm) to which is attached by cellophane tape a tube of cellulose acetate (also 9.5 $\times$ 2.5 cm) containing a platform of blotting paper. The end of the unit is closed with cotton and the vial contains 9.5 cc of a special uniform

cornmeal food to which has been added a measured quantity of yeast. Transfer of the populations to fresh feed is done on a weekly cycle of 2,2,2 and 1-day intervals. Flies hatching from the three vials in which the population stayed for two days each are collected every 2 (or 1) days, aged on unyeasted food, weighed, counted and added to the "front" population twice each week. The fourth vial in each week (1-day) is either discarded or the flies hatching therein used for samples. Fitness is thus measured by the biomass (wet weight of hatching adults) produced from the entire vial series during an arbitrarily chosen calendar week. Data are complete for 10 successive weeks for 3 populations of *D. robusta*, 1 of *D. americana* (Meramec Standard, Mo.) and 1 of *D. melanogaster* (*se ss k e^s ro*). *D. americana* produced a mean biomass of 178.8 ± 14.2 mgs/wk; the *robusta* populations produced 144.3 ± 10.8, 123.8 ± 6.2 and 107 ± 3.1 mgs/wk, respectively. Each of these latter is significantly less than *americana* and the lowest of the three (Plainwell, Mich.) has a fitness significantly less than that of the other two. The mean for *D. melanogaster* was 42.3 ± 5.2. The method appears to have promise as a means of assaying the microevolutionary capacity of different strains, races or species under various genetic conditions, to adapt under natural selection to specifically imposed laboratory conditions.

CASE, MARY E., and NORMAN H. GILES, Department of Botany, Josiah Willard Gibbs Research Laboratories, Yale University, New Haven, Connecticut: *Evidence from tetrad analysis for both normal crossing over and "gene conversion" at the pan-2 locus in Neurospora crassa.*—An extensive tetrad analysis has been made from a cross of two pantothenic acid mutants (B_3 and B_5) at the *pan-2* locus located in linkage group VI between the *ad-1* (3254) and *tryp-2* (75001) loci. These two mutants, although blocked in the same step in pantothenic acid biosynthesis (the conversion of keto-valine to keto-pantoic acid), complement to form a pantothenic acid-independent heterocaryon. When the mutants are crossed, this complementation test can be used to distinguish the segregants phenotypically. In extensive genetic tests using marked stocks, no separation of the heterocaryon-complementation pattern from the pantothenic acid requirement of either mutant has occurred. In an analysis of 937 tetrads utilizing a marked cross (*ylo ad-1$^+$ B_3 trypt-2* × *ylo$^+$ ad-1 B_5 trypt-2$^+$*) exceptional behavior at the *pan-2* locus was noted in 11 asci. On the basis of both heterocaryon and genetic tests, four different types of asci were obtained having the following isolate ratios: (1) two asci—1 wild type: 1B_3: 1 B_5 : 1 double B_3-B_5 ; (2) four asci—1 wild type: 1 B_5 : 2 B_3 ; (3) two asci—one wild type: 1 B_3 ; 2 B_5 ; and (4) three asci—1 B_5 : 3 B_3 . In the two asci of type (1) adjacent linked markers indicate that the B_3 and B_5 segregants are noncrossover (parental) types, while the wild types and B_3-B_5 doubles are reciprocal single crossover types. This evidence is taken to indicate that the *pan-2* "locus" can be separated into two units on the basis of genetic (crossover) as well as functional (heterocaryon) tests. The three types of atypical asci—with one wild type but no double B_3-B_5 or with a 3:1 ratio of mutant types—are interpreted at having arisen as a result of "gene conversion" affecting either one or the other or both parts of the *pan-2* "locus". Evidence that the types of "conversion" are related to the pattern of crossing over adjacent to the locus will be discussed. (This research was supported in part by contract AT(30-1)-972 with the Atomic Energy Commission.)

CASPAR, A., and W. R. SINGLETON, Blandy Experimental Farm, University of Virginia: *Induced "gene" mutation in maize.*—In 1953 and 1955 experiments were conducted to see if there were stages of maize microsporogenesis in which it would be possible to induce "gene" mutations. Plants carrying the dominant alleles were placed in Brookhaven gamma field for one or two days of radiation during different stages of maize microsporogenesis. Pollen was collected from all the plants used in the experiments on the same day and placed on silks of recessive tester stocks. Ears were harvested and scored for mutation from dominant to recessive.—The mutant seeds were planted and the F_1 plants selfed. There were 9 mutant endosperms recovered from 18,000 seeds produced from pollen which had received 50r/hour for 40 hours given in a two day period between 36 and 14 days before pollen was shed. 262 mutant endosperms were recovered from 7,100 seeds produced from pollen which had received the radiation for a two day period during the last 12 days of microsporogenesis. Five of the F_1 plants grown from the 9 mutant endosperm produced from the first group were homozygous

for the recessive mutant gene and produced ears without sterility. 130 of the 262 mutants produced in the second group were planted, 27 were homozygous recessive but in this case all the ears were semisterile.—From a control population of 108,000 seeds 81 mutant endosperms were recovered, when planted 11 of the F_1 plants were homozygous for the recessive gene and of these only one was not semisterile. We suspect that 3 of the mutants without sterility were prodjced from pollen that was radiated during one of the interphases preceding pollen mother cell formation and the other two from pollen radiated between meiosis II and the first microspore mitosis.—By selecting the proper stages of maize microspore development it is possible to induce changes which are inherited as simple recessive genes. (Work performed at Brookhaven National Laboratory and Blandy Experimental Farm. Supported in part by Contract AT-(40-1)-2071 between Blandy Experimental Farm, University of Virginia, and the U. S. Atomic Energy Commission.)

CHOVNICK, ARTHUR, University of Connecticut, Storrs, Connecticut: *Pseudoallelism at the garnet locus in Drosophila melanogaster.*—Recombination between mutant alleles at the garnet locus (chromosome 1-44.4) has been reported previously. Continued investigation has revealed three loci in the order g^2-g^{50e}-g^3. Two types of experiments are reported. (A) Examination of backcross offspring produced by females with free X chromosomes heterozygous for mutant alleles of garnet. (B) Examination of offspring produced by heterozygous attached-X females. In both groups of experiments, closely linked markers are used, and heterozygous autosomal inversions increase the crossover frequency in the garnet region.—From these studies, g^+ chromosomes, associated with recombination for closely linked markers have been recovered from all heterozygous combinations of garnet mutants. The behavior of marker genes indicates the described linkage order. In addition, g^+ chromosomes not associated with marker recombination have been recovered in all but one heterozygous combination. The pattern of origin and marker distribution suggests that such g^+ chromosomes result from spontaneous reverse mutation.—Double mutant chromosomes have been recovered from recombination in attached-X heterozygotes, and a cis-trans position effect noted. Thus, the genotype $g^2g^3/++$ exhibits the wild phenotype, while $g^2+/+g^3$ is mutant in phenotype.—Analysis of recombination data from attached-X heterozygotes, also heterozygous for various combinations of autosomal inversions reveals a clustering of X chromosomal crossovers associated with the increase in crossing over. These observations suggest that the role of inversions in increasing crossing over may involve an increase in premeiotic gonial crossing over.

CHU, ERNEST H. Y., and NORMAN H. GILES, Josiah Willard Gibbs Research Laboratories, Yale University, New Haven, Connecticut: *Chromosomal studies of normal and mutant clonal derivatives of human cell strains growing in vitro.*—The development by PUCK and his associates (1955, 1956) of efficient plating techniques for obtaining clonal lines of mammalian cells and their detection of phenotypically mutant clones in such materials are of considerable cytogenetical interest. The present abstract reports comparative chromosomal investigations of several human cell strains and their clonal derivatives. (These clonal lines were generously provided for these studies by DR. T. T. PUCK). The principal parental cell strain used, HeLa, originally isolated from a human cervical carcinoma, has been found to be cytologically abnormal in that its cells have different numbers of chromosomes with a peak frequency between 80 and 90, a conclusion in agreement with results reported by HSU (1954). The mutant clones derived directly from the HeLa strain (S1 and S3) and those derived from S3 (S3-R3, S3-RA1 and S3-RE1) have with one exception been found to be distinguisheble from the parental strain and from each other on the basis of either chromosome counts or karyotype analyses. All except S3-REI may be considered to possess a stemline condition accompanied by a marked reduction in chromosome number variation.—Additional observations on parental and non-mutant clonal derivatives from human liver, conjunctiva, and appendix cell strains (CHANG, 1954) have revealed that similar stem-line conditions exist in such clones in contrast to the much wider variation in chromosome numbers found in the parental lines. These results support the view that the clones examined in these studies consist of different pure lines derived from cytologically heterogeneous parental stocks. Furthermore, they indicate that differences in chromosomal constitution,

especially in chromosome number, must be considered, in addition to gene mutation, as mechanisms potentially responsible for the mutant phenotypes detected in such studies.

COHN, NORMAN S., Department of Botany, Josiah Willard Gibbs Research Laboratories, Yale University, New Haven, Connecticut: *The effect of carbon monoxide on the restitution of X-ray-induced chromosome breaks in Allium cepa.*—Previous studies (Genetics 41: 639, 1956) on the effect of fractionated doses of X-rays on the production of chromosomal aberrations in the root tips of *Allium cepa* have indicated that two classes of broken ends of chrosomes, as judged by their average restitution time, are induced by irradiation. The hypothesis was proposed that those breaks which undergo relatively rapid restitution (within 15 minutes) might be breaks of ionic bonds, while the more slowly rejoining breaks (which remain open for as long as 4 hours) might be breaks of covalent bonds in the chromosome.—Experiments were designed to test whether the two classes of breaks could be distinguished from each other by their responses to carbon monoxide. These experiments were based on the assumption that if the more rapidly rejoining breaks are ionic in nature, CO should produce no change in their radiation response, while if the more slowly rejoining breaks are breaks of covalent linkages CO would be expected to inhibit their restitution. In these experiments, the effect of CO was determined in pre- and postirradiation treatments and especially in treatments in which CO was present during the intervals of 5 min., 10 min., 1 hr., and 6 hrs. between two dose fractions. The results have shown that CO prevents the restitution of both the rapidly and the slowly rejoining breaks as long as CO is present in the cell, and that the effect of CO is light reversible. On the basis of the hypothesis that CO should affect the formation of covalent, but not of ionic bonds, such as Ca and Mg, the results provide evidence against the existence of ionic linkages in chromosomes. (This research was supported in part under contract AT(30-1)-872 with the Atomic Energy Commission, administered by NORMAN H. GILES).

CUANY, R. L., A. H. SPARROW, and VIRGINIA POND, Biology Department, Brookhaven National Laboratory, Upton, N. Y.: *Genetic response to Antirrhinum majus to acute and chronic plant irradiation.*—Clonal lines of snapdragon heterozygous for flower color have been irradiated with chronic gamma rays from cobalt-60 and acute X-rays. Pink or white flecks and sectors of various sizes on purple flowers are interpreted as somatic mutations induced at different times in flower development.—Acute X-ray treatment of preflowering stages caused no visible change in flowers opening prior to the 15th day after irradiation. Dense mottling on the 16th to 19th days was succeeded on the 20th to 21st day by numerous countable eight-cell mutant spots. The number of spots induced by 1000r gave a preliminary mutation rate estimate of 19×10^{-6} per cell per roentgen.—Chronic irradiation in the gamma-field and -greenhouse showed a linear response of mutation rate to daily dose rate, with seasonal variation of rates. There were occasional indications of nonlinearity but further tests have failed to show any significantly higher mutagenic efficiency of dose rates below 6r/day. —A comparison of the rates of production of small and large sectors by chronic and actue irradiation will be presented. The appearance of larger mutant sectors several months after exposure to either type of irradiation indicates the induction of persistent genetic changes. Progeny tests of seeds and cuttings are planned to test the nature of these genetic events. (Research carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.)

DE SERRES, FREDERICK J., Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee: *A genetic analysis of an insertional translocation involving the ad-3 region in Neurospora crassa.* —Previous studies have shown that the order of loci and map distances in the *ad-3* region on the right arm of LGI are as follows: centromere—0.5 units—*hist-2*—2.0 units—*ad-3A*—0.1 unit—*ad-3B* —3.0 units—*nic-2*. From a cross of a strain of *ad-3B* mutant B12 with a *hist-2 nic-2* strain, however, the proportion of histidine-requiring progeny approached 50% of the total adenine-independent progeny instead of the expected 3%. Since mature asci from this cross showed marked 6:2 and 4:4 abortion patterns, it appeared likely that mutant B12 was associated with some form of chromosomal rearrangement that segregated so that a large proportion of the *hist-2 nix-2* segregants carried a duplication covering the *nic-2* locus, and were thus histidine requiring. Crosses of B12 with strains

carrying other markers on the right arm of LGI showed that the duplication covered only the *ad-3B* and *nic-2* loci, and neither the *ad-3A* locus to the left, nor the *crisp*, *thi-1*, or *al-2* loci (located in that order) to the right of the *ad-3B* locus. Such evidence suggested that a chromosome segment carrying the *ad-3B* and *nic-2* loci had been deleted from the right arm of LGI and inserted in some other position in the genome. Crosses of B12 with *ad-3A* mutant A2 indicated that the distance from *ad-3A* to the proximal break point on LGI is about 0.1 map unit. Genetic data from crosses of B12 with LGI markers, combined with counts of the frequencies of various types of abortion patterns in mature asci, suggest that the deleted segment was inserted on some chromosome, other than the one carrying LGI, about 25 units from the centromere. Previous evidence demonstrating that the *ad-3* region is separable into two loci on the basis of heterokaryon and crossing analyses, is augmented by the present analysis indicating that the loci are separable by means of chromosome rearrangements.

DUBES, G. R., and M. CHAPIN, University of Kansas School of Medicine, Kansas City, Kansas: *Poliovirus mutants with altered responses to cystine.*—The poliovirus strains Akron (antigenic type I), Brooks (II), and Mabie (III) were previously shown to require exogenously supplied cystine for normal cytopathogenic action and propagation on monkey kidney tissue cultures. Aliquots of plaque-purified pools of these viruses were passaged ten times serially in monkey kidney tissue cultures maintained in a medium without cystine. The virus populations present in the various passage levels and plaque isolates therefrom were analyzed for their responses to cystine. These analyses revealed that at least three mutants of Akron virus, four of Brooks, and two of Mabie emerged during these passages. Plaque-purified pools of these were characterized by three criteria: (1) plating efficiency on kidney cultures without exogenously supplied cystine relative to efficiency with cystine, (2) plaque size on cultures with and without cystine, and (3) the fraction of intraplaque cells which are neutral red-stainable as a function of the concentration of exogenously supplied cystine. Thus the mutants have been tentatively symbolized as follows: *cr* (cystine-response), nonrequiring and uninhibited (Akron and Brooks); cr^{p}, partially nonrequiring and uninhibited (Brooks); cr^{si}, nonrequiring and slightly inhibited (Akron, Brooks, and Mabie); cr^{i}, nonrequiring and inhibited (Akron, Brooks, and Mabie). The original viruses are called $+^{cr}$ for convenience and are cystine requiring and not inhibited by cystine. The patterns of emergence of these mutants will be presented graphically. The possibility of extending this technique to include other "biochemical" markers will be discussed. (This research has been supported by the National Foundation for Infantile Paralysis.)

EBERSOLD, W. T., and R. P. LEVINE, Harvard University, Cambridge, Mass.: *Multiple strand crossing over in Chlamydomonas reinhardi.*—The observation has been made that from crosses involving two linked markers the frequency of nonparental ditype (NPD) tetrads is in excess over the expected number as calculated from the observed tetratype frequency. This result may be explained by assuming that 1) chromatid interference results in an excess of four-strand over three-strand double exchanges, or that 2) crossing over occasionally occurs at the two-strand stage. Our data indicate that the first alternative (chromatid interference) is responsible for the excess NPD tetrads. (This work was supported by grants from the United States Public Health Service, Grant No. E1421-C3, and from the National Science Foundation.)

ELLIS, J. F., Amherst College, Amherst, Mass.: *The effect of high hydrogen ion concentration on the growth and development of axenic Drosophila melanogaster larvae in whole casein media.*—During investigations on the growth requirements of axenic *In (2LR)40d* larvae, known to require ribonucleic acid (HINTON, ELLIS and NOYES 1951; HINTON 1955), tests were run utilizing media containing either whole casein or 13 amino acid bases as nitrogen sources at varying hydrogen ion concentrations. On a medium containing amino acids together with RNA and at a pH of 7.0, 58% of 150 initial larvae inoculated pupated in 13.2 median days from time of inoculation; of these pupae 92% eclosed in 17.3 median days. Similar results were obtained on the same medium but with hydrogen ion concentrations ranging from pH 8.0 to pH 4.0. On amino acid media lacking RNA, growth and development was obtained only when the pH of the medium was 7.0 (3.3% pupated of 300 larvae inoculated in 22.0 median days). When casein together with RNA was used at a pH of 5.0, 71.1%

of 270 initial larvae pupated in 6.4 median days; of these pupae 99.5% eclosed in 11.1 median days. On the same medium at pH 5.0 but without RNA, 67.1% of 240 larvae pupated in 10.1 median days; of these 88.8% eclosed in 14.5 median days. These results suggest that a utilizable precursor for RNA synthesis in the fly is present in media containing whole casein and is possibly produced by hydrolysis on autoclaving at a low pH. (This investigation was supported, in part, by a research grant E-1464, National Institute of Allergy and Infectious Diseases, Public Health Service.)

ELLIS, J. F., T. M. HARRIS, J. T. OSTHEIMER, and H. H. PLOUGH, Amherst College, Amherst, Mass.: *The effects of combined gamma radiation and ether treatment on the frequency of egg lethal in Drosophila melanogaster.*—Tests were made of the effects of 300r gamma from Co^{60} at 10r/minute, combined with 6–9 separate hourly etherizations given to young Samarkand males on the day of irradiation. Matings were made with fresh individual female sibs on each of six successive days following treatment. The frequency of nonhatching eggs was significantly decreased at the 1% level on the 3rd and 5th day following treatment as compared to irradiated controls that had received only 2 etherizations. The results of the 4th day were inconclusive; in 4 experiments out of 5 there was no significant difference between experimental and control series, in a single run the decrease in the ether treated series was significant at the 1% level.—In preliminary tests, 10 minute-old eggs, newly fertilized by sperm from males irradiated 4–1 hours earlier, were also tested after a 30 second treatment in a saturated ether atmosphere. At the 2% level of significance, these eggs showed a lowered frequency (15.3%) of nonhatchability over a control group whose males had received 300r but whose eggs were not etherized (19.8%). (Work supported by AEC Contract # AT (30-1)-930).

ENGLESBERG, ELLIS (introduced by BRUCE WALLACE), The Biological Laboratory, Long Island Biological Assn., Cold Spring Harbor, N. Y.: *Mutation to diauxie resistance.*—Mutants (c^-) of *Salmonella typhimurium*, strain LT2, have been isolated, which are able to use various Krebs cycle compounds but not glucose (or other carbohydrates), glycerol, or pyruvate as sole sources of carbon and energy. Although growth-factor amounts of aspartate and glutamate, as well as citrate, will bring about excellent growth with glycerol as sole carbon and energy source, there is negligible growth when glucose is substituted for glycerol. In fact, the addition of glucose (and certain other carbohydrates) to a medium containing citrate as the only carbon and energy source severely inhibits growth, and selection occurs for spontaneously occurring "glucose resistant" mutants (C^- dg^r). A study of the growth characteristics of the wild type (C^+ dg^s) and mutants c^- dg^s, C^- dg^r, and C^+ dg^r has established the following: (1) The wild type exhibits diauxie with glucose + citrate as carbon sources. (2) Mutation to glucose resistance of C^- dg^s is specific for the glucose inhibition. (3) The possession of this glucose resistance marker in an otherwise prototrophic genetic background leads to the abrogation of the glucose diauxie phenomenon, i.e., it leads to diauxie resistance to glucose + citrate and to other pairs of compounds containing glucose to which the wild type shows diauxie. (4) With wild type, unadapted to use citrate as the sole carbon and energy source, citrate still stimulates growth in the presence of "inhibitory" amounts of glucose. Diauxie resistance has so far been entirely refractory to all attempts at transduction. (Aided in part by grants from Office of Naval Research and National Microbiological Institute, National Institutes of Health.)

FABERGÉ, A. C., Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee: *Relation between chromatid-type and chromosome-type breakage-fusion-bridge cycles in maize endosperm.*—Two separate chromatid breakage-fusion-bridge cycles present in the same cell lineage could, in principle, convert to a single chromosome B.-F.-B. cycle. The reverse change is also conceivable. To test for this coalescence, the cross *I Sh Bz Wx Ds* × *Ds C Sh Bz Wx Ac/ac was* made. Such a kernel is in the first instance white, any colored areas showing that both female parent chromosmes had lost *I*, through breakage at *Ds* in standard position. The colored areas show twin spotting and mottling due to C-dosage, demonstrating that the male parent chromosome is undergoing a B.-F.-B. cycle generated at the distal *Ds*. This B.-F.-B. cycle, involving the markers *C Sh Bz Wx*, occurs in the presence of two broken ends in the female parent chromosomes, which presumably are themselves undergoing invisible B.-F.-B. cycles. Should the free end of the marked chromosome attach to an

unmarked arm, the double bridge could break so as to yield a loss of *Wx* without previous loss of *C*, or $L(C) \subseteq L(Wx)$. If the marked chromosome remains independent, the pattern can only be $L(C) \supseteq L(Wx)$. About 1700 kernels of the critical constitution were obtained, and no case of $L(Wx)$ underlying *C* was found. Tissue in which three homologous arms are broken is handicapped, but readily recognizable; the pattern is always $L(C) \supseteq L(Wx)$. Thus, in maize endosperm, two chromatid B.-F.-B. cycles do not coalesce to form a single chromosome B.-F.-B. cycle.

FLEISCHER, MARILYN L., ROBERT C. KING, and MORRIS H. HARNLY, Brookhaven National Laboratory, Upton, N. Y.; Cresap Laboratory, Northwestern University, Evanston, Ill., and New York University, N. Y.: *Oogenesis in adult Drosophila pseudoobscura.*—Oogenesis was staged from Kahle-Feulgen whole mounts of adult fly ovaries. Criteria used in demarcating stages included: (1) location and size of egg chambers, nurse cells and oocyte; (2) cytology of oocyte and nurse cells; (3) ratio of cytoplasm to nucleoplasm; (4) presence of border cells; (5) height of follicular epithelium; (6) development of micropyle, chorion and filaments.—Initially, the chambers are spherical; nurse cell chromosomes are thread-like or amorphous. Later, the cysts become ellipsoidal; yolk develops within the enlarging oocyte; the epithelium becomes columnar above the oocyte; border cells appear between nurse cells and oocyte. Finally, resorption of nurse cells occurs and accessory structures reach maximum development.—Fluctuations in stage distribution can be correlated with the periodicity in egg laying characteristic of this species. (Research carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.)

FLEISCHER, MARILYN L., ROBERT C. KING, and MORRIS H. HARNLY, Brookhaven National Laboratory, Upton, N. Y.; Cresap Laboratory, Northwestern University, Evanston, Ill. and New York University, N. Y.: *The effect of 2,4 dinitrophenol upon the uptake, localization and turnover of P_{32} in adult Drosophila pseudoobscura females.*—Newly emerged flies were placed on standard corn meal-molasses food to which tracer amounts of P_{32} and 2,4 dinitrophenol (0.0001 M, 0.0005 M and 0.001 M) were added. Females were dissected after varying intervals; the amount of P_{32} taken up by various tissues was determined.—Initially, most of the phosphorus is found in the gut and hemolymph; later, the ovaries and thorax have the highest percentage. Although females fed 0.0005 M and 0.001 M DNP incorporate less phosphorus, the distribution of phosphorus within the body appears normal if ovarian growth is comparable to that of the controls. However, ovaries from flies fed DNP seem to mature more slowly than ovaries from controls.—Upon being removed to unlabelled food, control females excrete phosphorus more rapidly. Thus, it is concluded that both phosphorus uptake and turnover are reduced in the presence of 0.0005 M qnd 0.001 M DNP. (Research carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.)

FLORY, W. S., JR., and THELMA F. SCHMIDHAUSER, The Blandy Experimental Farm, University of Virginia, Boyce, Va.: *Mitotic chromosome numbers in Hymenocallis with a consideration of factors possibly affecting numbers and karyotypes.*—Hymenocallis is a difficult genus from several standpoints. Plants of the group are not easy to collect, and are so succulent that many specimens found in herbaria are valueless with respect to critical characters. The genus is a dynamic one from the evolutionary standpoint. Many rather obviously distinct taxa still are difficult to separate by clear-cut character differences. Chromosome disparities are frequent in such cases. Of the 120 living accessions available for this study about 75 were collected in native habitats in the southern United States, Mexico or the West Indies. Species determinations are still lacking for many of these. Somatic chromosome numbers have been determined for 60 accessions. Numbers for Hymenocallis proper run from 38, through 40, 42, 44, 46, 48, 50, 52 and 54 to 69, 70, about 84, and 88 to 98. Additional numbers encountered in hybrids or in some representatives of the Ismene section are 45, 56, 74 to 80, with several taxa having 104 to 110 chromosomes. The number most frequently found is 46, and some taxa with higher numbers apparently trace back to 46-chromosome forms. Accessions with $2n = 40$ are next in frequency. Telocentric chromosomes occur in the complements of many taxa but have not been observed in those plants with either 46 (or 69), or 40, chromosomes. Most numbers other than 46 (or

69) or 40 reduce to one of these if half the number of telocentrics is added to the number of metacentrics. (Research supported by National Science Foundation Grant G2716.)

FORSTHOEFEL, P. F., University of Detroit, Detroit, Mich.: *Further studies on the effects of the luxoid gene on the skeleton of the mouse, including interactions with the luxate gene.*—Intercalary radial hemimelia combined with polydactyly occurs in about one luxoid homozygote in five. The os carpi radiale is absent or deficient and the radius is shortened slightly. In two luxoid homozygotes also heterozygous for the luxate gene, paraxial radial hemimelia combined with ectrodactyly was found. In these, the pollex was reduced to a vestige of its metacarpal; the ossa carpalia I and II and the os carpi radiale were missing; the radius was shortened and in one case lacked most of its shaft.—Reduction of the tibia combined with enlargement of the fibula is typical of luxoid homozygotes. Usually polydactyly is also found. In luxoid homozygotes also heterozygous for the luxate gene, the femur often loses its shaft; the tibia is very much reduced, sometimes completely absent; the fibula is deficient and bowed; the hallux is completely missing. In luxoid homozygotes also homozygous for the luxate gene the pubis has a deficient ramus; the epiphyses of the femur are much reduced; the fibula is deficient, sometimes forming only a spur on the os tarsi fibulare; the os tarsi intermedium is completely missing; ectrodactyly proceeds to the point where sometimes only two toes remain.—These results show that the luxoid and luxate genes act cumulatively on the hind limbs. Whether they act cumulatively on the forelimbs is doubtful. Probably the two cases mentioned above represent extreme effects of the luxoid gene in homozygous condition. (Supported by a grant from the National Science Foundation.)

FOX, ALLEN S., and JEAN B. BURNETT, Michigan State University, East Lansing, Mich.: *The components of the protyrosinase activation system in Neurospora crassa, strain 15300, and their production by genetically different cultures.*—Preliminary genetic analysis of the difference in tyrosinase activity and specificity between the mating types of strain 15300 (albino-2) suggested the existence of an enzyme precursor ("protyrosinase") (Fox 1954, Nature 173: 350). Subsequent work (Fox and BURNETT 1956, Genetics 41: 643) demonstrated that the tyrosinase content of homogenates of 15300-131a (a high activity, single-ascospore isolate from the cross 15300A × 15300a) increases during incubation, and that this activation process exhibits first-order kinetics which fit the model $P \rightarrow T$ or $P \rightarrow T + A$ (P, protyrosinase; T, tyrosinase; A, inactive byproduct). It has now been found that medium from 15300-131a cultures, when added to 15300-131a homogenates, increases the rate of activation and affects the equilibrium resulting in the production of an increased amount of tyrosinase, as if the medium contains protyrosinase. The effect is enhanced by dialysis and concentration by lyophilization. Paper electrophoresis discloses three proteins. Concentration by electroconvection of the component with the highest isoelectric point (pH 8.2) results in enhancement of the effect. Since the electrophoretic properties of the tyrosinase in 15300-131a homogenates is distinctly different, activation apparently involves a change in net charge of the molecule. Homogenates of 15300-138A (a low activity, single escospore isolate) exhibit no measurable activity before or after incubation. Medium from 15300-138A cultures, when added to 15300-131a homogenates, increases the rate of protyrosinase activation but does not affect the equilibrium. Dialysis and lyophilization enhances the effect, which therefore appears attributable to the production by 15300-138A of an enzyme catalyzing protyrosinase activation. The high tyrosinase activity of 15300-131a apparently results from production of both protyrosinase and activating enzyme, while 15300-138A produces activating enzyme but fails to produce protyrosinase. (Supported by grant no. C-2440 from the National Institutes of Health.)

FOX, ALLEN S., and SEI-BYUNG YOON, Michigan State University, East Lansing, Mich.: *Genetic mechanisms responsible for antigenic differences between males and females in Drosophila melanogaster.*—Antigenic extracts of adult, coisogenic XX females, XXY females, and XY sons of the latter have been tested with rabbit antisera by means of adaptations of Ouchterlony and Bjorklund agar-diffusion techniques. Such tests demonstrate a minimum of six antigenic components present alike in all three genotypes. In addition, tests of anti-XY sera demonstrate a component present in XY but not in XX or XXY, and tests of anti-XX and anti-XXY sera demonstrate two components present

in XX and XXY but not in XY. Tests of XO males are in progress and will be reported, but it seems probable that these components are not associated with presence or absence of the Y but with differences in number of X's. Another antigenic specificity is present in all three genotypes in a form capable of inducing antibody formation. As present in XX, it is also capable of precipitating the corresponding antibody, but in XXY and XY it inhibits the antibody without precipitation. The evidence suggests that this difference is due to alteration of the physical properties of the antigen, although quantitative differences are not excluded. Since the XY males are sons of the XXY females the effect is attributable either to matrocliny (maternal or cytoplasmic) or to the Y chromosome, while, the specificity of the antigen must be attributed to some other genetic mechanism. Tests of XO males and XY sons of XX females are in progress. The results, which should distinguish between matrocliny and Y-chromosomal effect, will be reported. (Aided by grants from the National Science Foundation and the National Institutes of Health C-2440).

FRYE, SARA H., (introduced by P. WEATHERWAX), Indiana University, Bloomington, Ind.: *Frequency of minute chromosome rearrangements in relation to X-ray dose in Drosophila melanogaster.*— Two findings (MULLER *et al.* 1934, 1935) which necessitated a study of the relation of minute rearrangements to X-ray dose are: (1.) that rearrangements with 2 breaks very close together occur with higher frequency than expected if breaks and unions occurred by chance (2.) the phenotypic stimulation of point mutations by minute rearrangements. Since studies by BELGOVSKY (1939) and by PANSHIN *et al.* (1946) regarding the relation of minute rearrangements to dose gave seemingly contradictory results the problem has been reinvestigated. As in BELGOVSKY'S work, the scute-8 chromosome, having heterochromatin close to the right of yellow$^+$ and achaete$^+$, was irradiated and "yellow mutations", usually representing small deficiencies or inversions (PROFOKYEVA 1938; RAFFEL 1939), were recorded. Males of composition *sc*^{8} *B*, 0–24 hrs. old were given 1000r or 4000r and these and simultaneous controls were mated to *y w In49 f* virgin-females (48–72 hrs. old). The frequencies of exceptional phenotypes among the F_1 Bar females were for the control, 1000r and 4000r treatments, respectively: "yellow" 0.00265% (7/263,694), 0.0791% (148/180,942), 0.3024% (126/41,310); "white" 0.0003% (1/263,694), 0.0051% (11/180,942), 0.0214% (9/41,310); "white-mottled" 0% (0/263,694), 0.0041% (9/180,942), 0.0311% (13/41,310); "forked" 0% (0/263, 694), 0.0025% (6/180,942), 0.0095% (4/41,310). These results show clearly that the yellow exceptions like gene mutations and unlike gross rearrangements, do not vary in frequency as a power of the dose higher than 1. A detailed genetic study of the yellow exceptions is now in progress to determine the relative frequencies of rearrangements of different kinds and sizes. (Work supported by grant to H. J. MULLER and associates from the American Cancer Society and the Atomic Energy Commission (Contract AT (11-1)-195).)

FUERST, ROBERT, and T. C. HSU, Division of Biology, The University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Texas: *Cytology of vegetative growth in Neurospora crassa.*— Employing slide cultures, phase micrography, and time-lapse cinematography, data have been collected on germination processes of the spores and cytology of vegetative growth in *Neurospora crassa*. The mean value for the diameter of conidial spores of several strains is very similar, but after 72 hours of incubation it increases with a rate characteristic for each strain. Wild type strains show only a slight increase of their spore diameter, whereas colonials may reach an enormous size many times that of the original value. In three strains analyzed, *Em5256A*, *Em5297a* and *T34A*, the time required for the germination of conidial spores is found to be inversely proportional to the spore size. The larger spores generally germinate earlier than the small ones, but the strains may differ in time requirement for a particular size class. Ascospores may germinate at one end or at both ends with a ratio of 2:1. When both ends germinate, they usually do so simultaneously. The protoplasm in growing hyphae is generally full of granules and vacuoles and streaming of protoplasm is exceedingly common. The nuclei are discernible only when the cytoplasm is relatively homogeneous and motionless. Mitochondria are in the form of dots or short rods; but when the adenineless strain *T34A* is grown in minimal medium, they become long filaments. Certain colonial mutants can be identified by their growth patterns, especially by the shape of microscopic colonies derived from single spores.

GARBER, E. D., The University of Chicago, Chicago, Ill.: *Genome analysis in the genus Collinsia*—The genus *Collinsia* (Scrophulariaceae) includes 21 recognized species, divided into 2 sections, depending on whether the flower is sessile (S genomes) or pedicelled (P genomes). The basic chromosome number is 7; no polyploid species have yet been found. The S genomes have a lower mean number of chiasmata per bivalent at MI than P genomes. Interspecific hybrids involving 4 species with S genomes displayed only bivalents; interspecific hybrids involving 2 species with P genomes had an interchange complex of 4 chromosomes and one of 6 chromosomes and 1 paracentric inversion; and interspecific hybrids between species with an S genome and a P genome displayed either 1 or 2 reciprocal translocations and 1 or 2 paracentric inversions and were completely sterile. An amphidiploid (PPSS) of an intersectional hybrid (PS) was fertile. It seems reasonable to assume that the P genomes have undergone a reorganization relative to the organization of the S genomes. Furthermore, the S genomes appear to have a common organization while the P genomes may differ in their organization. (Aided by grants from the National Science Foundation and the Abbott Memorial Fund, The University of Chicago.)

GARDNER, E. J., J. R. SIMMONS, and P. V. BLAIR, Utah State University, Logan, Utah: *Amino acids in tumorous head stock of Drosophila melanogaster.*—The present biochemical approach is designed to identify intermediate steps between genes and phenotypes and to discover a chemical basis for the maternal effect. Two types of experiments are in progress. One is carried out by adding amino acids to a standard medium and observing the effects on the penetrance and expressivity of flies with various genetic backgrounds. The other is a comparison of constituents in extracts of flies carrying different gene combinations, analyzed by means of paper chromatography.—When 0.5 percent tryptophan was added to a brewer's yeast-agar medium the penetrance of tumorous head was significantly reduced. Indole at .01 percent and DL-serine at 0.5 percent did not affect the penetrance while 0.1 percent L-cysteine significantly reduced the penetrance in the inbred stock but did not affect the results of outcrosses. The toxic effect of the chemicals may have been differential for flies of different genetic background.—Extracts of the body fluids of Canton, Stephenville and tumorous head flies were separated by two-diminesional paper chromatography using phenol and lutidine as solvents. Twenty ninhydrin positive substances were separated from Canton extracts. Eighteen of these substances were amino acids, but the other two remain unidentified. The chromatograms of the tumorous head flies differed qualitatively by the absence of unknown spot number two. The body fluids of tumorous head flies contained less cystine and proline. The concentration of alanine, asparatic acid, glutamic acid, methionine, tyrosine and unknown substance number one were higher in tumorous head than in the inbred wild stocks. (Supported by the American Cancer Society, Damon Runyon Memorial Fund and Utah State University Research Funds.)

GLASSMAN, E., H. S. FORREST*, and H. K. MITCHELL, California Institute of Technology, Pasadena, California: *Genetic control of xanthine dehydrogenase in Drosophila melanogaster.*—Crude extracts of two eye-color mutants of *D. melanogaster*, rosy (*ry*) and maroon-like (*ma-l*), have previously been reported as being deficient in xanthine dehydrogenase activity. This enzyme has now been purified approximately 80-fold from wild type flies, using charcoal, ammonium sulphate, and calcium phosphate gel. No activity could be detected in purified extracts of the mutants. Antibodies to xanthine dehydrogease were prepared by injection of purified extracts into rabbits. Preliminary data indicate that extracts prepared fron *ma-l* adults contain cross-reacting substances which remove the antibody to the enzyme, whereas *ry* is deficient in these substances. The relation of these data to theories of gene action will be discussed. (* The University of Texas, Austin, Texas.)

GOODGAL, SOL H., and ROGER M. HERRIOTT, Department of Biochemistry, Johns Hopkins School of Hygiene and Public Health, Baltimore, Maryland: *A study of linked transformations in Hemophilus influenzae.*—Transformation of sensitive *Hemophilus influenzae* has been obtained for a number of unlinked antibiotic sensitive characteristics and one pair of linked characteristics. DNA from the linked streptomycin-cathomycin resistant donor cells produced 100-times as many cells resistant to *both* antibiotics as were produced by a comparable mixture of unlinked factors, the latter correspond-

ing to the product of the individual frequencies. The kinetics of transformation of linked and unlinked markers supported the concept that double transformation produced by linked markers was the result of a single DNA-cell interaction; whereas, double transformation with mixed DNA's of unlinked markers resulted from the interaction of a cell with two molecules of DNA. A study of the effect of heat, deoxyribonuclease, and ultraviolet radiation on linked and unlinked markers have led to some ideas about the relative importance of certain structures of the nucleic acid for carrying genetic information.

GOODWIN, K., G. E. DICKERSON, and W. F. LAMOREUX, Kimber Farms, Inc. Niles, California: *A test of selection response in White Leghorn fowls.*—Genetic response to selection, environmental changes between generations (years), and changes in maternal effects associated with aging of the dams, have been measured in a closed population of noninbred White Leghorn fowls. The data consist of: a) intrayear comparisons of progeny from one-year old dams of two successive generations to estimate genetic changes; b) interyear comparisons of progeny from one-year old full sister dams of the same generation to estimate environmental changes; and c) intrayear comparisons of progeny from one-year old and two-year old full sister dams mated to the same sires, to estimate the change in maternal effects. The breeding scheme leading to such comparisons is based upon the repetition of identical matings during two successive years. These repeated matings are unselected, except as mortality may eliminate some.—Fifteen traits have been studied. Response generally has been in the expected direction during the two generations thus far studied, except that egg weight has declined in spite of mild selection for heavier eggs. Important changes in maternal effects from the time a dam is first used until she is again used as a two-year old have appeared for fertility, hatch of fertile eggs, percentage of cull chicks, and percentage of blood spots in the eggs. Environmental changes appear relatively important in measuring viability, number of eggs and egg quality traits.

GOWEN, JOHN W., SUI-TONG FUNG and JANICE STADLER, Iowa State College, Ames, Iowa: *Analyses of the contributions of the sex chromosomes, autosomes and a 3rd chromosome gene series to sex comb development in Drosophila.*—Three third chromosome genes in Drosophila, operating on the otherwise wild type background, influence sex expression. These genes are *Hr* of our stock, *tra* of STURTEVANT and the wild type at the locus. Chromosomal differences multiply possible types. One marked expression of these differences in the presence and development of the sex combs. The numbers of teeth on these combs furnish a quantitative measure of the degree of development of this element of sex. An equation to measure the quantitative effects may be constructed by considering the wild type of the *Hr* and *tra* genes as the base in the same manner as the base for dominance and recessiveness is established. The *Hr*/+ type may be considered as operating on the sex combs according to a quantity *a*, the *Hr*/+/+ to quantity *b*, *tra*/+ to *f*+*fh*, *tra*/*tra* to *2f*, *tra*/+/+ to *t*+*te*, *tra*/*tra*/+ to *2t*+*2mt*, *tra*/*tra*/*tra* to *3i*, the sex chromosomes (X) as *o* for one X, *p* + *pq* for 2X and *2p* for 3X and the autosomes as *o* for 2A and *r*+*rs* for 3A. By this formulation it is possible to measure the effect of the gene or chromosome separate from the other elements as well as direction and values of the dominance in heterozygotes or the 2X condition of the sex chromosomes. Eighteen genotypes have thus far been analyzed. The values of these constants show that the *Hr* gene is most effective in raising the number of sex comb teeth in the diploid somewhat less effective in the triploid. The *tra* gene has a lesser effect in the diploid and triploid and a large dominance coefficient acting toward a reduction in sex comb size. *Tra* acts in the same direction in the triploids but to a lesser amount. Increasing numbers of X chromosomes reduce the number of sex comb teeth and also show a negative dominance. Successive autosome sets increase sex comb teeth but their effects are quantitatively only about half as great as those of the *Hr*, or *tra* genes or the X chromosomes. (This work has received assistance from Contract No. AT(11-1)107 from the Atomic Energy Commission.)

GRAHN, D., and W. C. QUEVEDO, JR., Argonne National Laboratory, Lemont, Ill.: *Variability in the expression of tail-spotting in mice.*—LITTLE (1924) and GRÜNEBERG (1936) demonstrated that white tail-tipping in C57BL mice was due to a single recessive gene which exhibits incomplete penetrance. In the present study, reciprocal crosses of C57BL × BALB/c strains showed a decided ma-

ternal influence on the occurrence of white tail-spotting in the F_1 generation. Among C57BL♀ × BALB/c♂ (BC) F_1 progeny, 81.7% of 667 mice exhibited tail-spotting compared to approximately 90% in the C57BL strain. In the reciprocal cross (CBF_1), only 41.6% of 646 progeny showed tail-spotting. There was no evidence of sex linkage. Within each reciprocal there was a significant difference among dams as to the number of tail-spotted progeny and in both reciprocals there was an increasing incidence of tail-spotting with increasing parity (or maternal age). In F_2 offspring, the difference between reciprocal crosses was essentially eliminated (BCF_2 : 56.7% of 393; CBF_2 : 52.4% of 435). There was no demonstrable parity or dam effect in the F_2 generation. However, a significant difference in the incidence of tail-spotting was evident among the four coat-color segregants. This difference was primarily a function of the higher incidence among black mice (58.7% of 598) than among brown mice (45.3% of 232). Testcrosses involving C_3H mice (no tail-spotting) are being made to determine the relative contributions of C57BL and BALB/c mice to the occurrence of tail-spotting in the hybrid generations. (This work was performed under the auspices of the U. S. Atomic Energy Commission.)

GRELL, RHODA F., Calif. Institute of Technology, Pasadena, Cal.: *Non random assortment of nonhomologous chromosomes.*—Disjunction of the sex chromosomes and of the third and fourth chromosomes of *D. melanogaster* has been simultaneously studied by examining the progeny of XXY females heterozygous for a translocation between the third and fourth chromosomes—*T(3; 4)86D.* The seven chromosomes which have been followed are designated X, X, Y, 3, 4 and T, where T symbolizes the third and fourth chromosomes involved in the translocation. If the disjunction of the sex chromosomes and the third and fourth chromosomes were independent of one another, then the segregations X34-XYT and XT-XY34 should occur in a 1:1 ratio. The observed ratio, however, is 2.6:1. Similarly, the segregations X4T-XY3 and X3-XY4T should occur in a 1:1 ratio. The observed ratio is 20:1, based on the triplo-IV progeny, and is 17:1, based on haplo-IV progeny. Each of the above departures from a 1:1 expectation is statistically highly significant. (Other types of nondisjunctional products from this translocation do not lead to viable progeny. Progeny nondisjunctional for the X chromosomes constitute only 1.7% of the progeny and were not scored for the translocation). Stated in another way, the results indicate that, in females heterozygous for the above translocation, a normal Y chromosome tends to go to the opposite pole from the fourth chromosome much more often than expected on a random basis. This phenomenon is not observed in XYY males heterozygous for this translocation. (This work was supported in part by the Atomic Energy Commission.)

GROSS, S. R., Stanford University, Stanford, Calif.: *Enzymatic autoinduction in a mutant of Neurospora.*—A mutant (*arom*) of *Neurospora crassa* blocked in the conversion of dehydroshikimic acid to shikimic acid was found to have an impaired synthesis of dehydroshikimic acid reductase. In addition to the primary effect of gene mutation on the production of dehydroshikimic acid reductase the mutant produces two enzymes not found in extracts of wild type strains grown under comparable conditions. The two enzymes are: Dehydroshikimic acid dehydrase which catalyzes the conversion of dehydroshikimic acid to protocatechuic acid, and protocatechuic acid oxidase which catalyzes the oxidation of protocatechuic acid to β-carboxymuconic acid. The synthesis of protocatechuic acid oxidase can be induced in the wild type strain by the addition of protocatechuic acid or suitable in analogues to the growth medium. Since both dehydroshikimic acid and protocatechuic acid are accumulated by the mutant, the synthesis of both enzymes seems to be induced by the products accumulated as a consequence of an impaired biosynthetic sequence. The relation between autoinduced enzyme biosynthesis and the phenomenon of diauxie will be discussed.

HAAS, FELIX L., and CHARLES O. DOUDNEY, The University of Texas, M. D. Anderson Hospital and Tumor Institute, Texas Medical Center, Houston, Texas: *A relation of ribonucleic acid synthesis to radiation induced mutation frequency in bacteria.*—Numerous investigators utilizing various biological systems have reported that mutation frequency increases with radiation up to a certain dose level at which no further increase is observed with increasing dose.—We have recently found, using minimal grown cultures of *Escherichia coli*, that both frequency of mutation per unit dose and the

plateau level obtained with exposure to ultraviolet light may be increased considerably for certain mutations (modified glucose-galactose assimilation). This increase is obtained by short period incubations prior to irradiation in minimal medium supplemented with yeast extract, the purines and pyrimidines found in ribonucleic acid, or with riboflavin or p-aminobenzoic acid. Comparable results have been obtained with spot-checks using X-rays.—By utilizing cultures synchronized in cellular division the following has been established in regard to RNA purkne and pyrimidine influence: (1) maximum increase in the induced mutation level is attained during intervals of maximum RNA synthesis and several minutes prior to decreases of RNA synthesis associated with cellular division; (2) the level attained is reduced by cellular division; (3) cellular synthetic processes dependant on inorganic nitrogen and glucose must occur if the increased frequency is to be observed; (4) the increased mutation frequency observed is related to RNA synthesis since (a) all four purines and pyrimidines or their ribosides are required for maximum effect, (b) neither the purines and pyrimidines of DNA or their deoxyribosides show an appreciable effect; (5) the amount of RNA per cell is unimportant in determining mutation frequency; the relative capacity to synthesize RNA immediately after irradiation appears to be the important factor.

HECHT, ADOLPH, State College of Washington, Pullman, Wash.: *Incompatibility reactions in grafted styles.*—Pollen grains of *Oenothera organensis* were cut just above the juncture with the ovary and placed on moistened filter paper in covered culture dishes. The stigmas were dusted with either compatible or incompatible pollen of this species. After incubation for approximately 15 hours at 27°C it was found that whereas the incompatible pollen tubes had hardly penetrated the stigmas, those from the compatible pollen had grown an average of more than 100 mm from the base of the stigma. In further experiments the styles were cut and the cut ends fastened together with a gelatin-lactose mixture held in place by strips of lens paper. The pollen tubes frew readily through this graft juncture, reaching approximately the same lengths as did tubes in the uncut styles. When styles of different compatibility classes were grafted, pollen tubes that began growing in compatible styles continued to grow through a graft union for several centimeters into the incompatible styles. It has heretofore been assumed that the incompatible reaction is one involving specific inhibition of growth, and that this response occurs at any level in the stigma or style. If it can be shown that the reaction is no stronger in the stigma than the style, then an alternate hypothesis involving specific stimulation or the existence both of stimulation and inhibition is suggested. Further experiments are in progress in an attempt to check these hypotheses. (Work supported by funds provided for biological and medical research by the State of Washington Initiative No. 171.)

HERSKOWITZ, I. H., Indiana University, Bloomington, Ind.: *Intensity-independence of translocations from sperm X-rayed in females of D. melanogaster.*—Approximately 3360r were delivered to inseminated females at about 1900r/min either (A) continuously (in 1 min 45 sec) or (B) interruptedly (in 49 min 45 sec) in seven 15-sec irradiations given 8 min apart. The continuous treatment was also given to virgins subsequently mated with untreated males (C).—The II-III translocation rates for all F_1 males tested were 0% for C (no translocations in 422 tests), 14.3% for A (121/844), and 13.7% for B (135/982). Included here are counts for A and B of all F_1 males from eggs laid in the first 4 successive 4-day periods following irradiation. When individual 4-day periods were compared, the differences between results for A and B remained without significance (note following abstract). It is concluded that under these experimental conditions protracting the irradiation has no significant effect on translocation rate. (This work has been supported by a grant for work of H. J. MULLER and associates from the U. S. Atomic Energy Commission (Contract AT(11-1)-195).)

HERSKOWITZ, I. H., Indiana University, Bloomington, Ind.: *A relationship between translocation frequency and age at fertilization for sperm X-rayed in females of D. melanogaster.*—II-III translocation frequencies were determined in F_1 males from successive groups of eggs laid following treatment of inseminated females with approximately 3360r. For the first 4 consecutive 4-day oviposition periods after treatment the rates, combined for different experiments, were, respectively, 12.3% (111/906), 14.1% (203/1432), 16.8% (201/1194), and 19.7% (115/584). (The same dose given to

females before mating resulted in only one translocation in 1814 tests, hence the above translocations were paternal.) The translocation rate also increased in successive broods in each separate experiment even though the irradiation was delivered in different ways, continuously either at 1000r/min or 1900r/min, or discontinuously at 1900r/min, as described in the preceding abstract. (This work has been supported by a grant for work of H. J. MULLER and associates from the U. S. Atomic Energy Commission (Contract AT(11-1)-195).)

HERSKOWITZ, I. H., E. A. CARLSON, and H. J. MULLER, Indiana University, Bloomington, Ind.: *Sex-chromosome loss following X-radiation of D. melanogaster sperm.*—Males (12–24 hrs old), irradiated with 1200r (L) or 4800r (H) or untreated, were thereafter mated twice, each time for two days. The second mating employed phenotypically wild type females, homozygous for *y* in "snocty" attached-X's but carrying also y^+ $sc^{8\cdot}$Y:bw^+ (of COOPER) and homozygous *bw*. The males, likewise homozygous *bw*, carried a y^+-bearing non-ring X (normal or entire-inversion in arrangement) and one of the following Y's: (A) Y:bw^+ (Y^L marked), (B) y^+ $sc^{8\cdot}$Y:bw^+ (both arms marked), or (C) Y^c:bw^+ (marked ring). From the second mating, F_1 from eggs laid the first 13 days were scored for sex, eye and body color. For 43,132 control, 120,778 L and 38,370 H F_1 individuals the following percentages of *induced* (after subtracting control) sex-chromosome losses (partial or complete) were obtained from males with A, B, and C, respectively: after L, 0.18, 0.49 and 2.44; after H, 1.87, 2.80 and 6.67; H/L equalled 10.4, 5.7 and 2.7. For chromosome B, possessing two markers, 60% of losses were demonstrably partial. Its low H/L ratio as compared with A was doubtless caused by greater detectability of minute deficiencies. C, the ring, had much the highest rate of losses but the lowest H/L value, representing a sublinear relation of frequency to dose. This result presumably reflects a competition of other broken chromosomes for broken rings. This influence would be inappreciable if a high proportion of breaks of rings restituted. Breaks of nonrings, however, usually do restitute. Nonring losses are frequently multibreak, but of these many arise from one ion tract. (This work has been supported by a grant from the U. S. Atomic Energy Commission (Contract AT(11-1)-195).)

HEXTER, W. M., Amherst College, Amherst, Mass.: *Genetic resolution in Drosophila.*—The genetic material of microorganisms has been resolved by others into units of mutation, function and recombination, without agreement as to which (if any) unit is the "gene." An analysis of 4 *sn* alleles will be presented and the suggestion made that such complexity need not exist in Drosophila. The alleles fall into three phenotypic classes: sn^3; sn^2 and sn^4; and *sn*. They are separable into the same three classes by crossing over with linkage in the indicated order. Using the attached-X method the new data show 6 crossovers between sn^2 and *sn* in 43,326 tests, 4 between sn^4 and *sn* in 74,957 tests and none between sn^2 and sn^4 in 44,000 tests to data. All double mutants have resembled *sn*. In all singed tests to date 74 testable wild type females have been recovered from attached-X mothers with markers on each side of *sn*, only one of which failed to show that a recombination had occurred and which is reasonably explainable by causes other than negative interference. Although nothing is known of the function, at the gene level, of the *sn* alleles there is no compelling reason at present to interpret them other than as three genes each capable of mutation, function and recombination. It is suggested from these and other considerations that the organization of the genetic material in microorganisms and Drosophila is not the same. (Supported by research grants from the National Institutes of Health (RG-C-4599) and the Sigma Xi-RESA Research Fund.)

HINTON, TAYLOR, University of California, Los Angeles, California: *An analysis of naturally occurring lethals.*—Previous estimates of the number of loci in *Drosophila melanogaster* capable of mutating to lethality were made by determining the frequency of allelic lethals in a population. These studies neither established that the apparent alleles were different mutations nor that they were true alleles. To obtain more exacting data, collections of 2nd chromosome lethal stocks were obtained from 4 geographically widely separated populations: 23 different lethals from Pullman, Washington; 25 from Wooster, Ohio; 97 from Amherst, Massachusetts; and 63 from Blacksburg, Virginia. By crossing the stocks in the collections *inter se*, 14,366 crosses are possible. The results of about 5,000 of these crosses are reported here. The genetic analysis revealed 13 cases of allelism

between a lethal in one geographic population and a lethal in another. This would place the number of loci capable of mutating to lethality very close to the estimates previously derived by other workers. However, upon analysis by linkage studies and cytological examination of the salivary gland chromosomes, it has been shown that in few cases, if any, are these lethals true alleles. In some cases one "allele" is a deficiency, while its "allele" is a "point mutation"; in others, one is a position effect of an inversion, etc. This analysis leads to the conclusion that genetic tests alone are not reliable criteria for concluding that two lethals are mutations at the same locus, and furthermore suggests that an infinite number of cytogenetic situations can lead to lethality in Drosophila.

HOCHMAN, BENJAMIN, University of Utah, Salt Lake City, Utah: *Competition between wild type isoalleles in experimental populations of Drosophila melanogaster.*—Laboratory studies involving two wild type isoalleles at the fourth chromosome locus of cubitus interruptus in *Drosophila melanogaster* were carried out. The two alleles can be distinguished by their different degrees of dominance with respect to the mutant allele *ci*. One wild type allele, $^{+4}$, is completely dominant over *ci* while the other allele, $^{+3}$, allows a majority of flies with the genotype $^{+3}/ci$ to express the phenotype of interrupted wing veins which characterizes the *ci*/*ci* genetic constitution.—The population cage technique was utilized to determine the relative selective advantage of a chromosome carrying $^{+4}$ as opposed to one containing $^{+3}$. All flies were isogenic for chromosomes I, II and III dervied from a highly inbred strain. In each of four populations followed, $^{+4}$ increased significantly in frequency at the expense of $^{+3}$ regardless of the initial frequencies of the two chromosomes or the presence of an additional fourth chromosome carrying the recessives *ci* and ey^R. When populations were maintained for fifteen months $^{+4}$ rose to approximately 90 percent with no indication that an equilibrium had been attained.—Due to the apparent lack of adaptive heterosis indicated by mathematical analyses of the results, it appeared likely that $^{+3}$ would have been eliminated in time. If the difference in adaptive values between $^{+3}$ and $^{+4}$ determined under laboratory conditions is similar to that encountered in nature, rarity of ci^+ isoalleles of the $^{+3}$ type would be expected.

HOROWITZ, N. H., and M. FLING, California Institute of Technology, Pasadena, Calif.: *Further studies on the genetics of tyrosinase in Neurospora.*—We have described two naturally occurring forms of tyrosinase in *N. crassa* that differ in their thermostability, but not in various other properties. The thermostability difference is inherited as a single gene difference, one allele (T^S) determining the production of a thermostable form, the other (T^L) the production of a thermolabile form of the enzyme (Genetics 38: 360) Experiments designed to detect other variant forms of the enzyme are in progress. Preliminary results indicate a high degree of variability of the enzyme in nature. At this writing, only two properties—thermostability and electrophoretic migration—have been examined, but with respect to these, three new forms of the enzyme have been identified in wild strains. Some strains appear to be heterocaryotic, producing two distinguishable forms of tyrosinase which can be resolved by outcrossing. The mode of inheritance of the various modifications is under investigation. The following questions are of interest: Are all modifications of a given property—e.g., thermostability—referable to changes at the same locus? Does recombination between different properties of the same enzyme occur?—In addition to the above, a strain that produces no, or little, tyrosinase activity (kindly sent by M. WESTERGAARD) is being studied. This character appears to be inherited as a single gene effect which assorts independently of the *T* locus. This raises the question of whether these two genes act together in the synthesis of the protein part of the tyrosinase molecule, or whether, as one alternative, only one of them is so involved, the other being concerned with incorporation of the copper atom into tyrosinase.

HOVIN, ARNE W., University of California, Los Angeles, Calif.: *Seasonal variation of meiotic chromosome pairing in haploid Poa annua L.*—Response of pairing behavior of chromosomes to climatic temperature changes was studied at metaphase I in two haploid (2n = 14) perennial lines of *P. annua*. Fixation was made between 9:00 a.m. and 10:00 a.m. on six dates. When possible, at least 100 pollen mother cells (PMC) were tallied at each date for each line.—A significantly higher mean frequency of bivalents was recorded in warm than in cool weather. When these results were fitted to a linear

regression equation $y = a + bx$, it was found that line 1 fit the equation $y = .55 + .13x$ and line 2 fit the equation $y = 1.08 + .08x$ where y was the mean bivalents per PMC at each date of fixation and x was mean minimum night temperature for two nights preceding date of fixation.—Both regression coefficients were statistically significant, but there was no significant difference between them. In other words, there was a mean increase of one bivalent for an approximate 10°C increase in the mean minimum night temperature within the range of 7–16°C.

IINO, TETSUO, and J. LEDERBERG, University of Wisconsin, Madison, Wis.: *An analysis of monophasic variants of Salmonella.*—As reviewed in earlier studies (Genetics 41: 743–757, 1956) two genes, H_1 and H_2 control the specificity of flagellar protein in Salmonella serotypes. Which one is manifested in a given clone depends on an element closely linked or identical with the H_2 locus. Its alternation of state thus leads to the alternative expression which has long been known as 'diphasic variation' for these antigens.—Further mutations may be superimposed on this system to lead to *monophasic* variants, a number of which have been isolated from nature, and on at least one occasion in the laboratory. One group of variants has been analysed by transduction methods and proved to result from a mutation designated Ah_1^- which has the effect of blocking the synthesis of H_1 protein. When these cultures are in phase-1, they therefore fail to produce flagella and are immotile. In the sense that they oscillate between a nonmotile phase-1, which carries no flagellar protein, and a typical, motile phase-2, these cultures are still diphasic, but only one species of flagellar protein is produced.—Three separable Ah_1^- mutants have been identified, all closely linked to H_1 and Fla_1 and presumably therefore to one another. As far as can be judged by the absence of trails that might come from abortive heterogenotes, these mutants are noncomplementary, i.e. belong to a single cistron.—The Ah_1^- effect should be distinguished from that of Fla^- which suppress flagella of either phase.—A number of other classes of monophasic variants have been found. All the types are consistent with a biosynthetic scheme containing common links (*Fla*) and a switch point controlled by the phase-determinant at H_2.

IVES, P. T., Amherst College, Amherst, Mass.: *Mutation frequencies in successive batches of irradiated D. melanogaster sperm.*—Oregon-R males, collected young and aged 2 days without females, were irradiated with Co^{60} gamma and then mated singly to successive groups of 3 females daily for 18 days. The females carried the seven recessive *hes* mutants. The F_1 were scored for *hes*, Minute and other bilateral abnormal phenotypes in tests of 100r, 1000r, 5000r and 10,000r starting with 16 irradiated males at each dose. The results from 46,725 F_1 flies suggest that the mutation frequency is relatively constant at each dose in the sperm of days 1–4 but that it changes differentially with dose thereafter. A five-fold increase over the frequency in days 1–4 appeared only in days 5 and 6 at 100r but extended through day 8 at 1000r. A "sterile" (no offspring) period limited to days 9 and 10 at 1000r extended through days 5–10 at 5000r and 5–14 at 10,000r but was absent at 100r. After the sterile period at 1000r and 10,000r and after day 6 at 100r the mutation frequency did not differ quantitatively from controls which were limited to days 1–3, but at 5000r the frequency in days 11 and 12 seemed to be like that in days 1–4, after which it dropped to the control level. A 500r test is in progress as are similar 18 day tests of the sex-linked lethal mutation rate at doses of 250r, 500r, 750r and 1000r.

JAAP, R. G., MARION W. MURRAY, and R. W. TEMPLE, Ohio State University, Columbus 10, Ohio: *Genetic effects on variance in hormone response of the comb and testes of young male chickens.*—In view of the genetic effects on the variance in testis weight of day-old chickens (JAAP and THOMSON, 1944, Growth 8: 311) and on comb weight (JAAP and ROBERTSON, 1953, Endocrinology 53: 512), estimates of the additive and nonadditive genetic effects have been secured for males produced from diallel matings within a random bred flock closed from crossbred ancestry. The data include those from day-old and eleven-day controls as well as two hormone treated groups. The combs of one group were treated with sesame oil containing 0.5 mg of testosterone propionate per ml in six daily applications from the fourth to the ninth day. The other hormone treated group received two Cartland-Nelson units of pregnant mare serum gonadotropin in five daily subcutaneous injections

from the fifth to the ninth day. Maternal effects on comb and testis weights are high in the day-old chick. Nonadditive genetic effects account for 20 to 30 percent of the total genetic variance. By eleven days after hatching maternal effects tend to disappear but nonadditive genetic effects on comb weight remain high. Genetic response to direct androgen stimulation of the comb is mostly additive and heritability is high. Variation between diallel sets is great and additional data are being collected to improve these estimations.

JAKWAY, J. S., R. W. GOY, W. C. YOUNG, and J. A. WEIR, University of Kansas, Lawrence, Kans.: *Inheritance of reproductive behavioral patterns in male and female guinea pigs.*—Pattern of reproductive behavior in inbred strains 2 and 13 differs for both males and females. Eighteen adult males from each strain, 20 F_1, 30 F_2 and 50 backcrosses to each parent strain were scored with an estrus female during 7 weekly observation periods. Eighteen females from each strain, 23 F_1, 47 F_2, 48 backcrosses to strain 2 and 55 backcrosses to strain 13, were spayed and observed during estrus induced with two levels of α-estradiol benzoate (100 and 50 I.U.) followed by 0.2 I.U. progesterone.—For males phenotypic array according to sex score suggests dominance of the mating pattern of lower scoring strain 13. This pattern is distorted by the F_1 scores which exceed those of the higher scoring parent strain 2. The high F_1 score is attributable to more frequent ejaculation than either parent strain. For the mounting component of the score, phenotypic array shows dominance of the less active strain 13.—Of the parent strains, strain 13 has significantly greater body weight and lower adrenal weight. Both characteristics show dominance in inheritance.—For females, at 100 I.U. α-estradiol benzoate, the pattern characteristic of strain 2 estrus, (short latency, long duration, short maximum lordosis, and few mounts) shows phenotypic dominance in inheritance. All F_1s and 75% of F_2s resemble the parent strain 2. At 50 I.U. α-estradiol benzoate, more strain 2s than strain 13s come into estrus. F_1s and F_2s are intermediately responsive.—Analysis of backcrosses is continuing for females as data accumulates. (Supported by research grant M-504(C4), from the National Institute of Mental Health of the National Institutes of Health, Public Health Service.)

JAMES, ALLEN P., Atomic Energy of Canada, Ltd., Chalk River, Ontario: *The process of nongenic mutation in an unstable strain of yeast.*—The spontaneous production of respiratory-deficient mutants of nongenic origin was investigated in an unstable strain of *Saccharomyces cerevisiae*. Experimental methods included timed measurements of the size of microcolonies to permit a determination of the relative degree of instability of individual cells. The mutation was found to be lethal but death can be averted by a subsequent gene mutation. Stability of the strain is correlated with rate of division of cells. Evidence is presented that the appearance of a mutant is the result of a sequence of chance events rather than of the breakdown of a system involving the orderly assortment of cytoplasmic factors.

JANICK, JULES, D. L. MAHONEY, and E. C. STEVENSON, Purdue University, Lafayette, Indiana: *Cytological analysis of diploid × triploid crosses in spinach.*—Cytological analyses on diploid pistillate × triploid staminate crosses with spinach (*Spinacia oleracea* L.) have revealed what appears to be a unique type of segregation. While there was real variation from cross to cross, of 558 plants from such crosses; 51% were diploid (12 chromosomes), 19% were trisomic (13 chromosomes), 1% had 14 chromosomes, 1% had 16 chromosomes, 12% had 17 chromosomes, and 15% were triploid (18 chromosomes). There were no plants found from this type of cross with 15 chromosomes, although 15 chromosome seedlings were found from the reciprocal cross. The trisomics fell into six morphological groups which probably represent the six possible trisomics (n = 6). Segregation for sex in crosses of diploid pistillate × triploid staminate plants is a function of whether the staminate parent is duplex (XYY) or simplex (XXY) for the factor conditioning sex determination.

JUDD, B. H., University of Texas, Austin, Texas: *Complex pseudoallelism at the white locus in Drosophila melanogaster.*—Studies of recombination between various mutants of the white series show the white region to be very complex. In addition to the two pseudoallelic loci demonstrated by LEWIS (1952) and MACKENDRICK and PONTECORVO (1952) and possibly a third (white-spotted,

LEWIS 1956), another, white-Brownex (w^{Bwx}), has definitely been located to the left of w^a. Among the offspring from $w^{Bwx}/y^2w^a spl\ ec$ free-X females (heterozygous for autosomal inversions) are found the types $y^2\ w^+$ and $y^2\ w$ with a frequency of about 0.02 percent. Attached-X studies have shown that the class complementary to $y^2\ w^+$ (i.e., $w^{Bwx}\ w^a\ spl\ ec$) does occur but was not recovered from the free-X studies because its phenotype is similar to w^a. The origin of the $y^2\ w$ class is as yet unexplained.—Results similar to these have been obtained from w^{Bwx}/w^h and w^{Bwx}/w^{bl} free-X females. w^h has been definitely placed at the right of w^a, but w^{bl} has not been located since $w^{bl}/y^2\ w^a\ spl\ ec$ free-X females give $y^2\ w$ and $w\ spl\ ec$ crossover classes.—w^{Bwx} does not show recombination with w^{bl} and in turn w^{bl} does not recombine with w^a, but, as pointed out above, w^{Bwx} does crossover with w^a.—In one experiment $y^2\ w^a$-*like spl*, $y^2\ w\ spl$ and w types arose from $w^{bl}/y^2\ w^{bl}\ spl$ free-X females with a frequency of 2, 2 and 4 respectively in about 29,000 offspring. Attempts to repeat these results have not been successful, however, the stocks established from each type are being studied in an effort to determine how they arose.—The complex spatial relationships of some of the mutants of the white series will be discussed.

KALTER, H., The Children's Hospital Research Foundation, Cincinnati 29, Ohio: *Further evidence of the association between maternal weight and frequency of cleft palate in the offspring of cortisone-treated pregnant female mice.*—In two previous crosses (Am. J. Physiol. 185: 65, 1956; J. Exp. Zool. 134: 1, 1957), maternal age and weight were closely correlated and statistical analysis by multiple regression was required to evaluate the effect of each of these maternal attributes on the frequency of cortisone-induced cleft palate. By this method, maternal weight alone was shown to be associated, inversely, with the frequency of the defect. It was also found that, although the average frequency of the defect was very different in the two crosses, the degree of protection provided by maternal weight was of the same order in both.—These findings have been substantiated by a third type of cross [(DBA/1♀ × C57BL/6♂)♀ × DBA/1♂], in which, however, maternal age and weight were not correlated. Thus, a simple regression was here sufficient to show that weight was associated with cleft palate frequency ($b_1 = -.020144$, $P < .01$), but that age was not ($b_2 = .000318$, $P = .7 - .8$). The average frequency of cleft palate ($46/102 = 45.1\%$) was different from those in the previous crosses, but the regression coefficient again was not different ($t = 1.055$, $P = .3$). The uniformity of this finding may indicate that the level of protection afforded by maternal weight against cortisone-induced cleft palate in mice is constant.

KAMRA, OM P. (Introduced by D. U. GERSTEL), Dept. of Field Crops, North Carolina State College, Raleigh: *Effects of polyploidy on cotton fibers.*—Changes of fiber properties, and particularly of tensile strength, are an important aspect of interspecific hybridization in cotton. The effects of artificial polyploidy employed in the hybridization program introduce a complicating factor. In this study the effects of doubling of the chromosomes on fiber perimeters and on the X-ray angles of the cellulose deposit were investigated. Colchicine induced polyploids of New World cottons (*Gossypium hirsutum* and *G. barbadense*) and Old World species (*G. herbaceum*, *G. arboreum* and their F_1 hybrid) were produced. Some of the treated plants were chimerical affording an opportunity to determine the developmental relation between the lint bearing seed epidermis and leaf epidermis. In some individuals seed and leaf epidermis were both found to be doubled, while in other cases only one or the other had polyploid features, suggesting their derivation from distinct germ layers. Fiber perimeters were significantly increased in Old World tetraploids as compared with their diploids. With one exception, in *G. hirsutum* octoploids the fiber perimeter was not increased; this was ascribed to the chimerical condition of the plants rather than to an upper limit for cell size since 8n *G. barbadense* fibers did have an enlarged perimeter. For any particular genotypes and their derived polyploids the data revealed distinctly proportional changes of X-ray angle and mean perimeter, leaving the number of helical turns of the cellulose deposit uniform per unit length of fiber. On the other hand, since the length of the helical turn varies from species to species, and can be made subject to selective change within a species, it is tentatively suggested that this characteristic is directly dependent upon the genotype.

KAPLAN, WILLIAM D., and BENJAMIN HOCHMAN, Division of Research, City of Hope Medical Center, Duarte, California: *The influence of ethylene and acetylene upon the rate of mutation induced in D. melanogaster by X-irradiation.*—The strong reducing agents, ethylene and acetylene, are known to combine readily with peroxides. Adult male flies were irradiated in an atmosphere of ethylene or acetylene in order to measure the effect that the removal of peroxides, at the time of irradiation, might have upon the mutation rate. A significant reduction in the frequency of dominant lethals, but no parallel decrease in the rate of sex-linked recessives, has been found as a result of this experimental treatment. The influence of these chemicals upon a system in which it has been shown that peroxides play a significant role is to be studied. (Supported by a grant from the Atomic Energy Commission).

KAPLAN, WILLIAM D., BENJAMIN HOCHMAN, and JOSEPH T. HOLDEN, Division of Research, City of Hope Medical Center, Duarte, California: *Patterns of free amino acids in D. melanogaster.*—A study by means of two-dimensional paper chromatography has been made of the free amino acid patterns of different life stages of wild type and several mutant strains of *D. melanogaster* before and after X-irradiation. Strain, male and female, and different life stage patterns have been compared. Amino acids occurring in high concentration are ethanolamine phosphate, glutamic acid, taurine, alanine, β alanine, glutamine, and proline. Lesser quantities of glutathione, the leucines, histidine, arginine, asparagine, lysine, aspartic acid, cystine, valine, tyrosine, serine and glycine have been found. A difference in the amount of a substance tentatively identified by chromatographic techniques as methionine has been found to exist between males and females. The level of this substance is higher in females than in males and is apparently intermediate in XO males. Data on the identification and quantitative determination of this substance will be presented. (Supported by a grant from the Atomic Energy Commission.)

KING, R. C., Northwestern University, Evanston, Illinois: *The radiation-induced ovarian tumors of Drosophila melanogaster.*—Ovarian tumors are induced in the fruit fly by ionizing radiation. A dose of 4000r of Co^{60} gamma rays produces an incidence of tumors 26 $\times$ the control rate. Whenever two or more tumors occur in the same ovariole they are adjacent to one another. This finding argues for a clonal origin of the tumors. A mechanism of tumor formation is postulated. It assumes that in a specific cytoplasmic zone of the germarium there is localized a chemical which stimulates a portion of the genome of the cells which enter the region. The stimulated genetic material then manufactures a substance which gradually inhibits in a precise fashion further cytokinesis. Radiation occasionally inactivates that portion of the genome of an oogonium responsible for the production of the inhibitor. This mutation is passed to the progeny of the oogonium, and tumors develop as a consequence. (Work supported by the U.S.A.E.C.)

KIRCHNER, C., and A. EISENSTARK, Kansas State College, Manhattan, Kansas: *The influence of divalent cations on frequency of recombination in E. coli K12 and transduction in S. typhimurium.*—Increases in frequency of recombination and transduction have been observed upon treatment of appropriate bacterial cells with versene or upon propagation in media deficient in divalent cations. The effects were more pronounced upon divalent ion starvation than upon treatment by the chelating agent, versene. Increases in numbers of prototrophs occurred only when recipient cells were treated, never when donor cells were treated. In *S. typhimurium* transduction, the response was not the same at all loci; increases were observed in the transduction of certain markers but not in others. Since the increases in numbers of prototrophs were attributed to the removal of divalent ions, and thus to increase in crossing over frequencies, these ions were added after treatment of cells to observe reversal consequences. Calcium was more effective than magnesium in the reversal response. Addition of manganese to cells that had been starved of divalent ions resulted in even higher frequencies of transduction, indicating that manganese could spare an ion essential for genetic stability.—While the experimental results are compatible with the hypothesis that divalent cations bridge genetic units in *E. coli K12* and *S. typhimurium*, a search has been made for alternative explanations such as

changes in physiological state or surface properties of recipient cells as a consequence of ionic disturbance.

KOSIN, I. L., State College of Washington, Pullman, Wash.: *Genetic variability of feathering and growth in domestic turkeys.*—410 pedigreed Broad Breasted Bronze poults (189 males and 221 females, offspring of 20 sires and 80 dams) were weighed individually at 16, 18, 20, 22, 24 and 26 weeks. Also, pinfeather counts were made on the breast and on the ventral patagial area. Because of Poisson distribution, the counts were transformed to $\sqrt{X + 3/8}$. Estimates of genetic and environmental variances and covariances were made for weights and counts by the method of intraclass analysis. The estimate of choice involved the combined components of sire and dam.—In males the heritability for body weight was only half of that of females in which it consistently surpassed 0.5. Heritability of breast counts stabilized, in the males, at < 0.6 but in the females it was low, $>$ 0.3 except at 24 and 26 weeks. For wing counts the reverse was true: h^2 tended to decrease with age in both sexes.—High genetic correlations among body weight *gains* (< 0.5) were general and, of course, among the weights proper. Genetic correlations among pinfeather counts within and between locations were rather variable, particularly in the latter growth stages. The same was true for rg between counts and weights. Much of this undoubtedly was due to inherent large sampling errors. Environmental correlations, in general, were less variable in their distribution and of low order of magnitude.—Results suggest that (1) selection, on individual basis, for improved body growth and feathering should be effective, (2) same genes appear to control growth between 16–26-weeks, (3) same genes are involved in determining feathering during late growth period, (4) body growth and feathering genetically are not correlated traits.

LAUGHNAN, J. R., University of Illinois, Urbana, Illinois: *The origin of noncrossover derivatives from certain A^b: Peru complexes in maize.*—Several A^b (beta:alpha) alleles of Peruvian origin yield spontaneous noncrossover and crossover alpha derivatives with relatively high frequency (ca. 1/1000) and for this reason were chosen for an intensive analysis of the possibility that the noncrossover alpha derivatives may result from gene mutation of the beta element to a null level. The following observations suggest that these derivatives are not ascribable to gene mutation: (1) mutations of the isolated beta element, measured in the A^b/beta heterozygote, are far less frequent than expected; (2) if gene mutation of beta is responsible for the occurrence of noncrossover alpha derivatives, the latter should carry a null element in the beta position in the complex. Preliminary studies designed to isolate the hypothetical null element have given negative results; (3) recent experiments suggest that the beta element of these A^b complexes of Peruvian origin is itself a complex of at least two elements separable by crossing over. Thus the spontaneous occurrence of noncrossover alpha derivatives from these complexes would require the simultaneous mutation of more than one element.—Gene mutation, gene conversion, inhibition based on a Ds-Ac-like system, and multiple crossovers in short segments have been suggested to account for rather similar cases in Aspergillus and Neurospora. These and other possibilities are considered in the light of evidence indicating that the A^b complexes are duplications.

LEFEVRE, G., and A. C. BARTLETT, University of Utah, Salt Lake City, Utah: *Incidence of mutants following irradiation of Drosophila melanogaster females.*—Drosophila females carrying the reversible forked allele f^{3a} were exposed to an X-ray dose of 4,200r delivered at the rate of 400r per minute. After irradiation, the females were mated individually to $y^2\ w^a\ v\ f$ males (carrying inversions) and subcultured at 2^- or 3^- day intervals over a period of 4 weeks. The incidence of recessive sex-linked lethals, Minutes, and forked reversals was followed. With more than 80,000 F_1 progeny examined and 7,000 F_2 cultures raised, the incidence of each class of mutants appears to follow a different trend with time. The incidence of sex-linked lethals was highest in the first culture (being equivalent to that found in the first cultures of males exposed simultaneously to the same dose), dropped to approximately half the initial value by the second week, leveled off there until the fourth week when some increase was noted. Minutes declined sharply in the first week, then progressively decreased more slowly until by the fourth week virtually no Minutes were found. Forked reversals exhibited a

rather constant incidence throughout the experiment. (None were found after comparable exposures of males during the first 2 weeks after irradiation.) Thus, the more deleterious a mutation is in the heterozygous condition (at least as judged by its effect on the adult), the more unlikely it is to be found among progeny produced by irradiated females as time passes following the exposure.

LEVINE, M., and R. CURTISS, Biology Department, Brookhaven National Laboratory, Upton, L. I., N. Y.: *Genetic studies with mutants of phage P22 affecting lysogenization of Salmonella typhimurium.*—The spontaneous clear-plaque mutants of the temperate phage P22 of *Salmonella typhimurium* can be classified into at least three phenotypic groups c_1, c_2 and c_3. The mutants of group c^h are temperate, being able to lysogenize. The mutants of groups c_1 and c_2 behave effectively as virulent phages, producing lytic responses, lysogenizing little or not at all. Mixed infection with any mutant c_1 and any mutant c_2 results in lysogenization of sensitive bacteria at frequencies similar to those obtained with the wild type temperate phage. The lysogenic bacteria arising from $c_1 - c_2$ mixed infections carry prophages of the c_1 type. Recombination studies have been carried out with both normal and ultraviolet irradiated stocks of these mutants. Ultraviolet treatment of phage before infection is known to increase genetic recombination. The genetic sites of these mutants fal into a circumscribed region of the phage linkage mag consisting of four pseudoallelic series or functional units. The c_1 and c_2 mutants can be arranged into two distinct and nonoverlapping regions. The c_3 mutants can also be divided into two distinct regions (separated by the c_2 unit) and it is probable that the mutants of the two regions may be phenotypically distinguishable. The four pseudoallelic series are closely linked and can be arranged in a linear order as can the individual mutations in each series. TAYLOR's suggestion that the DNA of chromosomes might be arranged as specifically attached side chains on a backbone has not been experimentally substantiated in P22 if a functional unit is equated to a side chain. (Research carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.)

LEVINE, R. P., and W. T. EBERSOLD, Harvard University, Cambridge, Mass.: *Studies on crossing over in Chlamydomonas reinhardi.*—Experiments directed toward the investigation of the mechanism of crossing over are being carried out in *C. reinhardi*. A number of authors have presented data which suggest that metallic ions play a dominant role in the crossover process in several organisms. To date, a study of the effects of calcium and magnesium deficiencies and excesses have shown that these ions do not alter the frequency of crossing over between the two linked loci arginine-1 and arginine-2. Furthermore, the chelating agent EDTA was found to have no effect on recombination frequency. It is suggested, therefore, that metal ions play little if any role in crossing over in *C. reinhardi*. A second series of experiments is concerned with the relationship between the time of crossing over and the time of DNA synthesis. Its purpose is to ascertain whether or not the crossover process is in any way associated with DNA replication. Data concerning the relationship between these two phenomena will be presented. (This work was supported by grants from the United States Public Health Service [Grant No. E1421-C3] and from the National Science Foundation.)

LEWIS, GARY B. (introduced by M. T. M. RIZKI), Reed College, Portland, Oregon: *Further mutability of a white variant of Serratia marcescens.*—Diffusible precursors of red pigment of some variants of *Serratia marcescens* (Hy strain) can induce phenotypic color alterations in other variants. Utilizing the method reported by RIZKI, a white variant *W40* was characterized in terms of its ability to produce color changes in five other variants, or in turn, be altered by the presence of these variants. Suspensions of *W40* cells were irradiated with ultraviolet, and the colonies representing the surviving cells were plated with the five test variants. Any change in the original interaction pattern of *W40* with any of these variants was classified as a mutation of *W40*. The mutation frequency of *W40* is 2.3% (that of the Hy strain under comparable conditions, 50.3%) demonstrating the usefulness of this method for detecting mutations in white variants of Serratia.

LINDEGREN, C. C., and G. LINDEGREN, Southern Illinois University, Carbondale, Ill.: *The effects of constantly occuring localized crossovers and polarized centromeres on tetrad analysis in Sac-*

charomyces.—In tetrad analysis, linkage is defined as deviation from a 1:1:4 ratio of tetrad types I, II, and III and is expressed in five fundamental distributions: (1) N, 1:1:4, (2) L,>1:<1:<4, (3) R, <1:>1:<4, (4) F, 1:1: <4 and (5) T, 1:1: 4. L-distributions (conventional linkage) occur in all progeny of hybrids between certain genes: (1) the triplet of genes controlling the syntheses of anthranilic acid, isoleucine and histidine, (2) the pair of genes controlling the fermentation of galactose and resistance to anisomycin and (3) the pair controlling synthesis of methyl-α-D-glucopyranoside and resistance to cadmium.—F-distributions, in which tetratype is less than unity, and R-distributions are unequivocal evidence that the genes involved are linked to the respective centromeres of two different chromosomes. A pair of genes which occur in an R-distribution in one hybrid may be expected to occur in an L-distribution in another depending upon whether the centromeres are preferentially segregated to the same or opposite poles. The same genes sometime occur in F-distributions in still other hybrids.—An extreme T-distribution is evidence for the occurrence of a localized crossover at every meiosis between the pair of genes involved. The constantly occurring crossover is characterized by its tendency to shift in position along the chromosome and to occur in different regions in specific hybrids. A T-distribution may be superimposed upon L-, F- or R-distributions. Pedigrees demonstrating deviations in tetrad-distributions of the progeny of different hybrids will be described.

LINDSLEY, DAN L., and C. W. EDINGTON, Biology Division, Oak Ridge National Laboratory Oak Ridge, Tennessee: *Sex-linked recessive lethals whose expression is determined by the Y chromosome.*—The high rate of primary nondisjunction characteristic of *In (1) sc*^{4L}, *sc*^{8R} has been incorporated into a modification of the Muller-5 technique that allows detection of induced sex-linked recessive lethal changes depending of either the presence of the Y or the absence of the Y for their expression. *y*/Y males are irradiated and crossed to M-5/M-5 (*sc*^{4}*B InS w*^{a} *sc*^{8}) females; the resultant M-5/*y** (* = irradiated X) females are crossed individually to S-5/*sc*^{8}.Y (*y sc*^{4} *B InS w*^{a} *sc*^{8}/*sc*^{8}.Y$_{2}$) males. The types of zygotes formed by fertilization of *y**-bearing egg nuclei and their approximate frequencies are as follows: *y**/*sc*^{8}.Y = + ♂, 0.35; *y**/0 = *y* ♂, 0.15; *y**/S-5 = *yB* ♀, 0.45; and *y**/S-5/*sc*^{8}.Y = *B* ♀, 0.05. Nonlethal cultures yield both + males and *y* males, wheras lethal cultures most commonly lack both of these male classes. Of interest are those cultures from which *y* males only or + males only are missing. The former are relatively common and are lethals whose expression is suppressed by the Y chromosome; it might be anticipated that this class contains many lethals in the *bb* region. If this were true, the *y B* female class (*y**/S-5) should also be absent foom the F_2, since S-5 is deficient for much of the proximal heterochromatin; however, *y B* females were generally present in cultures from which *y* males were absent. Cultures from which only + males were absent represent lethals that depend on the presence of the Y chromosome for their expression; this class is very uncommon. These three classes of lethals have been compared genetically.

LIVINGSTON, C. A., and G. YERGANIAN, The Children's Cancer Research Foundation, and Harvard Medical School, Boston, Massachusetts: *Hypotetraploidy and new chromosomes in sublines of tumor CH-38MC of the Chinese Hamster.*—Three sublines of tumor CH-38MC have shifted to hypotetraploid levels from the original hyperdiploid modal frequency of 24 chromosomes. Two of these sublines were characterized by the presence of new chromosomes in as many as 90 percent of the cells examined. Increased polyploidization has failed to drastically alter immunogenetic relations of the host and tumor, as previously reported for the mouse (HAUSCHKA *et al.* Ann. N. Y. Acad. Sci. 63: 683, 1956). Three new chromosome forms were present in a frozen subline stored as −76°F. At present, only one large rod-shaped type is persisting. The form in the second subline is very similar and approximately one micron longer. Comparison of the four new types with the basic 11 forms and their aberrancies induced by alkylating agents (TEM and Nitromin) strongly indicate that all have arisen from pericentric breaks of Chromosome I. Such deletions are lethal when induced or occur spontaneously in hyperdiploids. However, this expression is masked when occurring spontaneously in hypotetraploids. Thus, tumor cells, in the act of shifting to the tetraploid range, may retain detrimental mutations that would otherwise have led to the elimination of the parental cell. (This investigation was supported in part by a research grant, C-2860, from the National Cancer

Institute, U. S. Public Health Service, and grant # 2930 of the Damon Runyon Memorial Fund for Cancer Research, Inc.)

MARTIN, ALBERT JR., Veterans Administration Neuropsychiatric Hospital, Pittsburgh 6, Pa.: *Geographic endemicism in relation to antibiotic therapy.*—Endemic strains of bacteria resistant to many or all currently employed antibiotic and chemotherapeutic agents are the end result of persistent attack *in vivo* of such drug therapy where the agent employed does not rapidly and readily destroy the invading organism. The transition from sensitive to resistant seems to be genetic selection for it is not rapid but fluctuates in a natural selection sequence over a relatively long period of time; three years in this study, utilizing 200 cultures of genito-urinary origin, involving 30 different patients and over 2,000 antibiotic sensitivity evaluations. Inhibition and/or stimulation of growth and multiplication have been observed in the following genera: Proteus, Pseudomonas, Escherichia, Paracolobactrum, Klebsiella and Alkoligenes; species strains of the latter two genera are stimulated in growth and multiplication in the presence of Furadantin and Penicillin respectively, while species strains of the other genera studied exhibit a shift in inhibition from one group of antibiotic to another. Once strain endemicism of a genetic order has obtained, it is possible, in diagnostic bacteriology, to employ developed inhibition and/or stimulation as an aid in species identification. Sensitivity testing provides a means, tenuous to be sure, for observing evolution in action through the *in vitro* and thereby *in vitro* development of bacterial strains whose resistant reactions change as the degree of endemicism increases. This dynamic process points up the concept, based on intelligent interpretation, that genotypic adaptation as a dynamic force must be entertained in the total attack on those organisms pathogenic to man.

MEYER, HELEN U., Indiana University, Bloomington, Ind.: *Frequencies of lethals and chromosome losses after irradiation of Drosphila pole cells with ultraviolet and X-rays.*—Posterior ends of Drosophila embryos at polar cap stage were exposed to 2537 Å ultraviolet (300 ergs/mm^2), or 200 KVP X-rays (1500–2000r), while the major (somatic) portions were shielded. The female embryos had attached-X chromosomes and either a ring-Y (Y^{Lc}) or a V-shaped Y (*sc*.Y^{L}), both types being irradiated simultaneously. Sons of female imagines developing from these embryos were tested for presence of the maternal Y, while sons of males were tested for lethals in chromosome 2. Survival of treated zygotes to adulthood was lower for X-ray (6.1%) than ultraviolet groups (9.2%), probably because of less effective X-ray shielding. That at these doses ultraviolet affected somewhat more pole cells than X-rays is indicated by the larger size of "clusters" presumably of common origin (reciprocally related to germ cell survival) after ultraviolet than after X-rays. Further indication of this is given by losses of *sc*.Y^{L}: ultraviolet (17/1640) 1.0 ± .4%, X-rays (4/731) .55 ± .3% controls (9/4209) .21 ± .07%, where errors allow for clustering. However, with Y^{Lc} the high control rate and low productivity obscure the relations: ultraviolet (20/462) 4.3 ± 1.5%, X-ray (13/232) 5.6 ± 3.6%, control (90/2767) 3.2 ± .5%. Pooled lethal frequencies were: ultraviolet (73/1321) 5.5 ± 1.7%, X-rays (20/883) 2.3 ± 1.0%, controls (12/2507) .41 ± .14%. Thus for this stage ultraviolet is the more practicable chromosome and gene mutagen. Cluster sizes for lethals (ultraviolet 17.9%, X-rays 10.2%, controls 4.9%) exceeded those from losses (respectively 5.8%, 4.4%, 2.7%), despite male embryos (source of lethals) having more germ cells than females (source of losses). This indicates that losses were effectuated after more delay. (This work was supported by a grant to H. J. MULLER and associates from the American Cancer Society.)

MILLER, W. J., and C. STORMONT, University of California, Davis, California: *Parentage exclusions by blood grouping in dairy cattle.*—Mating is rarely random in cattle. Consequently, theroretical estimates of the likelihood of settling parentage problems by blood grouping tend to exaggerate success. Nevertheless, it was found in this laboratory that 95 percent of the problems submitted by member organizations of the Purebred Dairy Cattle Association during the past two years were solved. This value is based on 250 parentage cases. The usual problem was that of determining which one of two or more bulls qualified as the sire of a calf by a known dam. Such a case was considered solved when only one bull was shown to qualify. The blood type of the calf was examined

for those phenogroups in each of 11 blood-group systems which must have been transmitted by the paternal parent alone.—The B system, controlled by well over 100 known alleles, was critically involved in the solution of more than 50 percent of the cases solved; the C system in 25 percent; each of the six systems F-V, Z, S-U, A-H, L and J between 5 and 10 percent; and the H′ and Z′ systems in less than 2 percent. The highly frequent phenogroup D of the D system was not critically involved in these cases. Only one system was critically involved in the solution of half of the cases although the qualifying bull usually had to qualify in one or more additional systems in which the excluded bull or bulls qualified.—Ten percent of the cases submitted would not have been solved by the factor method alone, but were solved by the method of phenogrouping.

MOREE, RAY, VERNE F. NEWHOUSE, and JAMES R. KING, State College of Washington, Pullman, Wash.: *Studies on the relative viability of the mutant ebony*^{11} *of Drosophila melanogaster.*—When parents and progeny of the cross $+/e^{11} \times +/e^{11}$ are maintained under crowded conditions, the number of mutant progeny surviving decreases as the degree of crowding increases. With minimum crowding, viability is 99.9%, with maximum, 25.8%. This decrease in viability of the mutant progeny occurs in spite of the fact that crowding has lowered parental fecundity. Such lowering of parental fecundity evidently is not sufficient to prevent crowding of the progeny. Testcrosses show that the frequency of $+/e^{11}$ does not vary with crowding. Its frequency from pooled data being 68.5%, $+/e^{11}$ is slightly but significantly heterotic. Results of the crowding experiments demonstrate that the major component of inviability is environmental and that it increases with population density. To test whether such inviability results from crowding among the larvae themselves, or from the effect of the crowded parents upon the larvae, experiments were devised in which the density of adult and larval populations were varied with respect to each other. Such experiments permit the division of the environmental component into two parts, one due to larval crowding, the other to parental adult crowding. Results show the larval effect to about 40 times greater that the adult. In some experiments adult crowding actually causes a slight, though not always significant, increase in viability. Inviability of the mutant, therefore, is chiefly a consequence of larval crowding and a resulting competition for food. (Work supported by funds from the State of Washington Initiative Measure No. 171 for the support of biological and medical research, and by grant RG-4174 of the National Institutes of Health, Public Health Service.)

NEWCOMER, EARL H., University of Connecticut, Storrs, Conn.: *The status of the micro-chromosomes in the domestic fowl.*—A study of mitosis in the fowl with and without chromosomal pretreatments revealed the so-called microchromosomes to be not chromosomes but what are here defined as chromatoid bodies. They apparently arise in early prophase from chromocentric heterochromatin; they are variably allocyclic throughout mitosis and disappear prior to the eu-chromosomes in each succeeding telophase. Analogous behavior is shown for them in meiosis and it is suggested that they function as reserves of nucleoproteins for the chromosomes, and if genetic, only in a quantitative or modifying capacity.

OAKBERG, E. F., and R. L. DIMINNO, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee: *X-ray sensitivity of different stages of primary spermatocytes of the mouse.*—Since irradiated primary spermatocytes frequently degenerate during meiotic division, quantitative estimates of the survival of these cells are essential for adequate interpretation of genetic effects observed in the offspring from successive weeks of mating during the presterile period of irradiated male mice. Two hundred and twenty-four hybrid (101 × C3H) male mice were evenly distributed among partial-body exposures of 0, 100, 200, 300, 400, 600, 800, and 1000r of 250-kv X-rays. Sufficient time was allowed for completion of meiosis and degeneration of damaged spermatocytes before newly formed spermatids were counted. Intervals at which mice were killed were selected to indicate survival of cells irradiated in preleptotene, leptotene, zygotene, early pachytene, middle pachytene, late pachytene, diplotene, and diakinesis to metaphase I. Comparison of experimental/control ratios indicate a significant effect of meiotic prophase stage on survival. Frequency of anisocytosis among spermatids also was dependent upon stage irradiated. Since differences in nuclear size prob-

ably arise from aneuploidy, the data will be discussed in relation to frequency of normal division figures at anaphase I and II. From the combined data on survival and chromosome breakage, it should be possible to estimate the contribution of irradiated primary spermatocytes in gamete formation for any radiation dose up to 1000r of X-rays.

OSTER, I. I., Indiana University, Bloomington, Ind.: *Suggested mechanism underlying the differential radiosensitivity of cells having condensed chromosomes.*—Although the condensed state of the chromosomes (such as is found in spermatozoa and late prophase, metaphase, and anaphase of mitosis and meiosis) of many organisms, including Allium, Trillium, Ascaris, Drosophila, and Rana, is the most radiosensitive, results (OSTER, 1956, Radiobiology Symposium) of a comparison of the radiosensitivity of *Drosophila melanogaster* spermatids and spermatozoa, both having condensed chromosomes, have indicated that some other factor(s) may be responsible for the greater sensitivity of the former cells. In the hope of elucidating the basis for this differential sensitivity, X-irradiation in nitrogen, air, and oxygen of spermatids, mature spermatozoa in 3–4-day-old males, and mature spermatozoa contained in 2-day-old females with 1200r, 2800r, and 2800r (175kV, 400r/minute) respectively, was carried out. Autosomal translocation tests of spermatids treated in nitrogen yielded 1.7% (24/1416), treated in air 14.2% (46/324), and treated in oxygen 19.5% (33/196). Translocation frequencies obtained from spermatozoa in the male and female, respectively, were 2.7% (34/1239) and 4.2% (48/1137) when X-rayed in nitrogen; 6.4% (25/389) and 10.2% (42/413) when X-rayed in air; and 17.2% (53/309) and 19.8% (41/207) when X-rayed in oxygen. The fact that the data indicate a relatively greater effect of reducing the oxygen tension and a relatively lesser effect of increasing the oxygen tension on the radiosensitivity of spermatids as compared to the other two stages also having condensed chromosomes lends support to the suggestion (OSTER, *ibid.*) that the high sensitivity of spermatids may be due to more intra- and/or intercellular oxygen being normally present (or available) in these cells. (This work was supported by a grant to DR. H. J. MULLER and associates from the U. S. Atomic Energy Commission [Contract AT (11-1)-195] and by a postdoctoral fellowship from the National Science Foundation.)

PALIWAL, R. L., and B. B. HYDE, University of Oklahoma, Norman, Oklahoma: *The effect of calcium and magnesium on chiasma frequency in Plantago ovata.*—EVERSOLE and TATUS (1956) have shown that in Chlamydomonas treatment of cells with EDTA brought about an increase in crossing over which was associated with a decrease in the Ca and Mg level of the cells. To determine directly the effect of these cations on chiasma frequency in plants, *Plantago ovata* seedlings were raised in glazed pots containing pure quartz sand and fed the following different nutrient media: (1) HOAGLAND and ARNON'S complete medium; (2) with Ca increased five times; (3) with Mg increased ten times; (4) deficient in Ca; (5) deficient in Mg. Since it is not possible to grow *P. ovata* with complete deficiency of Ca or Mg from the very beginning, the plants were raised on complete medium for about five weeks and then were switched to medium completely deficient in Ca or Mg. The buds were collected from these plants when symptoms of Ca or Mg deficiency were clear. *P. ovata* is a fairly good plant for estimation of chiasma frequency since it has only four pairs of chromosomes, all of which have blocks of centromeric heterochromatin. The chiasma frequency was estimated by counting the number of rod or ring bivalents per cell at metaphase I. Plants deficient in Ca showed a highly significant increase in the chiasma frequency. The deficiency of Mg also increased the chiasma formation but the effect was less marked than with Ca deficiency. The excess of Mg had no significant effect. On the other hand, the excess of Ca, also, showed a highly significant tendency towards increasing the number of chiasmata. (This work was supported by a grant [#RG-4675] from Public Health Service National Institute of Health.)

PARER, D. R., University of Mississippi, University, Miss.: *Radiation induction of compound X's in Drosophila oocytes.*—It has been suggested that dominant lethals may be induced in Drosophila oocytes by breakage of two sister chromatids, followed by sister union, giving rise to Anaphase II bridges. PARKER (1955) assumes independent breakage of chromatids in young (stage 7) oocytes, isochromatid breakage in old (stage 14) oocytes, accounting for the change from "two-hit" to "one-

hit" response, while KING (1957) concludes breakage is isochromatid in both cases, but with two or more AII bridges being necessary for lethality in stage 7. Similar events occurring in an inversion heterozygote, with appropriate crossing over, might give rise to reversed acrocentric compound-X chromosomes. Irradiation of females heterozygous for the sc^8 inversion (*sc^8 w^a cv v f/ y car*) has produced a number of compound X's. In stage 7 oocytes, about half of those induced are homozygous either for *f* or for *car*; in stage 14, nearly all are homozygous for one of these markers. Limited data are consistent with a "two-hit" response in stage 7 and "one-hit" in stage 14. KING's interpretation requires absence of an intensity effect on dominant lethals in both stages. In fractionation experiments, a divided dose gives significantly higher survival in stage 7, while no such effect is found in stage 14. (This work was supported by contract AT-(40-1)-2163 with the U. S. Atomic Energy Commission.)

PARTRIDGE, C. W. H., NORMA J. NELSON, and NORMAN H. GILES, Department of Botany, Josiah Willard Gibbs Research Laboratories, Yale University, New Haven, Connecticut: *Genetical restoration of a specific enzyme activity in Neurospora.*—As part of an investigation of the genetic control of adenylosuccinase formation by the *ad-4* locus, a number of adenine-independent reversions have been derived from several members of a series of 21 adenineless mutants at this locus. Back mutants induced in conidia by ultraviolet as well as prototrophs derived from interallelic crosses are under examination for their quantitative levels of adenylosuccinase activity and for qualitative differences in the properties of the enzyme. In a series of 15 induced back mutants from a single allele, none was found to exceed a specific activity (on a dry weight basis) of 55% that of the parental wild type. In contrast, among 12 examples of adenine prototrophs derived from 4 different interallelic crosses specific activities relative to wild type ranged from 75% to 125%. Mutant crosses from which these prototrophs were derived carried linked markers on either side of the *ad-4* locus, and the prototrophs tested included representatives from each of the four progeny types with respect to markers—the two parental and the two reciprocal crossover categories. Tetrad crossing analyses have been carried out to determine the degree to which individual specific activities are characteristic of the state of the *ad-4* locus in particular back mutants induced in conidia. To date, an examination has been made of six complete asci from crosses of two different back mutants with a parental wild type strain marked at a closely linked locus. In all asci examined clear 2:2 segregations were observed between marked wild type and back mutant strains, the latter possessing specific activities about one half those of wild types. The significance of these observations in interpreting mechanisms of back mutation in conidia as contrasted with those of "reversions" at meiosis will be discussed. (This research was supported in part by contract AT(30-1)-872 with the Atomic Energy Commission.)

PERRY, T. O., and C. W. WANG, University of Florida, Gainesville, Florida: *Genetic variation in the winter chilling requirement of red maple (Acer rubrum).*—Red maple (*Acer rubrum*) grows in an area ranging from northern Canada to the Everglades National Park in southern Florida. The frost free season in this area ranges between 365 and less than 80 days.—Collections of red maple seedlings from various points in this range were subjected to varying amounts of cold treatment. Genetic variation in the amount of cold required for normal break of dormancy was clearly demonstrated as a result of these experiments. Plants failing to receive an adequate amount of cold treatment did not break dormancy in a normal manner. Plants from the Everglades National Park in Florida have no chilling requirement and break dormancy without cold treatment. The winter chilling requirement is evidently controlled by several genes or alleles and the pattern of genetic variation is clinal and is correlated with the frost free season where the seed was collected.—Observations from horticulture indicate that this clinal variation in winter chilling requirement may be characteristic of most north temperate woody plant genera and serves as a limiting factor in moving many horticultural varieties southward. Pink dogwood, peaches, many varieties of pears and blueberries, and so forth, cannot be raised in the mild climate.

PETERSON, PETER A., Iowa State College, Ames, Iowa: *A new mutable at the A_1 locus in maize (a_1^m).*—During the course of our studies with a mutable pale green gene (pg^m) (1953 Abstracts),

two new mutables were observed in one plant growing in the field in 1952. One was a mutable waxy (wx^m)-chromosome 9 and the other a mutable *a* (a_1^m)-chromosome 3. These new mutables represented changes of the dominant alleles, *Wx* and A_1, to their unstable recessive forms, wx^m and a_1^m, respectively. Only one, $a_1^{m_1}$, was crossed for further tests. The unstable allele a_1^m is characterized by a change from the colorless (a_1) to the anthocyanin colored condition (A_1) in the kernel. This results in a variegated condition. Different patterns of variegation are recognized which are heritable as definite properties of the individual a_1^m allele that is mutable. Tests of recoverable germinal mutants to A_1 reveal that the new A_1 is stable. a_1^m also mutates to a completely colorless, stable condition-a^s. These new a^s mutants occur both as individual germinal mutants and as somatic sectors on the ear. The rate of change from a_1^m to a_1^s varies among the different patterns. There have been no observable reversions of a^s to the mutable condition.—Since the a_1^m arose in the pale green mutable stocks, crosses were made test to whether the two mutables, pg^m and a_1^m, are governed by the same mutability system. The introduction of a_1^m into pg^s stock (pg^s—stable since it lacks the mutator, *En*) failed to cause pg^s to become mutable suggesting that a_1^m lacks the mutator *En* and that mutability of the two mutable genes is governed by different systems. a_1^m does not require the dotted gene, *DT*, to be mutable. a_1^m was also crossed to DR. MCCLINTOCK'S *Ac* tester. No evidence of *Ac* activity was obtained. Although the mutability of a_1^m seems to be autonomously controlled, the possibility of a separable, independently segregating factor is being further investigated. A number of parallels exist in the behavior of a_1^m and pg^m.

PHILLIPS, J. H., W. BRAUN, and O. PLESCIA, Institute of Microbiology, Rutgers, The State University, New Brunswick, N. J.: *Studies on the antigenicity of bacterial DNA*.—In the past the demonstration of the antigenicity of deoxyribonucleic acid (DNA) has met with little or unconvincing success. In view of the genetic implications of any demonstrable DNA-specific antibodies and the availability of a different procedure for the isolation of highly polymerized DNA from bacterial cells, the problem has been reinvestigated. The DNA was isolated from *Brucella abortus* cells by extraction with 0.5% phenol in citrate saline and antisera against deproteinized preparations, as well as against whole donor cells, were produced in rabbits.—A study of the resulting antisera has included a variety of techniques, including inactivation of certain biological phenomena mediated by DNA and immunochemical procedures such as precipitin and gel diffusion techniques. The most striking results so far have been obtained with the latter method which showed the presence of a Feulgen positive antigen-antibody complex. Details of these observations, the techniques, and their implications for genetic and immunochemical studies will be discussed. (This work was supported in part by NSF Grant G-2184 and USPHS Grant E-1137 during the senior author's tenure as a Waksman-Merck postdoctoral fellow.)

PITTMAN, D., and C. C. LINDEGREN, Southern Illinois University, Carbondale, Ill.: *Implications from radiation studies on the fine structure of the gene*.—Ultraviolet and X-rays studies, in conjunction with tetrad analysis, on the degradation and regeneration of the melezitose gene (*TMSGZ*) in Saccharomyces indicate that the gene occurs in at least seven configurations, each of differing sensitivity to ultraviolet. Degradation of the *TMSGZ* gene by ultraviolet is sequential (*TMSGz*, *TMSgz* . . . *tmsgz*) rather than random, while regeneration of the *tmsgz* allele by X-rays is abrupt (*TMSGZ*) rather than stepwise. The degraded alleles induced by X-rays thus far have been principally *TMSgz* and *tmsgz*. The *tmsgz* allele is refractile to back mutation by ultraviolet but can be reversed by X-rays. The observed radiation effect, coupled with genetic studies, can be interpreted as an intragenic phenomenon.—The ultraviolet-induced alleles of the gene undergo gene conversion in the heterozygous state, acting as donor or recipient of a specific genetic pattern or configuration, depending upon the allelic state. BEADLE'S hypothesis of miscopying cannot explain the 0:4 ratios observed in genetic analyses of such crosses. The occurrence of induced mutations at 3 loci (*TMSGZ*, *SU*, *MA*) in cells plated on nonselective media indicate that the radiation effect extends over segments of the chromosome or is controlled by postradiation substrates.—These findings indicate that radiation-induced reorganization of the fine structure of the gene to the dominant state is not random and can be controlled by specific postradiation substrates.

PLAINE, H. L., Ohio State University, Columbus, Ohio: *A sex-differential suppressor of erupt in the Swedish-b strain of D. melanogaster.*—Regardless of the strain tested (of those having the suppressor-erupt system), or of the nature of obtaining the erupt phenotype, no differences between males and females have been obtained in the frequency or expression or erupt eyes. One wild type strain, Swedish-b, spontaneously produced a few erupt flies and a rigorous program of selection for this phenotype was started. The expression is always more extreme in the females and in every generation there was a significant difference between the low frequencies of affected males and the high frequencies of affected females, until the females reached maxium frequency (98–100%); then the males, in successive generations, increased to 99% affected.—In a substrain selected against the phenotype, however, the difference between affected females and males increased so that the former were 3 to 4 times as frequent as the latter. From chromosome substitution tests, it is apparent that the suppressor, or at least the second chromosome, is responsible for allowing the mutant to become expressed and also for the sex difference in frequency. When the Swedish-b second chromosome is made homozygous in either the erupt (*Su-er*$^+$; *er*) or suppressor-erupt (*Su-er*; *er*) strains, the same sex difference appears. Homozygous substitutions of other Swedish-b chromosomes do not affect the two sexes differently. In reciprocal crosses the sex difference is greater when the Swedish-b second chromosome comes from the female parent.—There is some indication that the X chromosome may carry a modifier of erupt, but it does not act differently in the two sexes.

RAPER, J. R., MARGERY G. BAXTER, and GLADYS S. KRONGELB, Harvard University, Cambridge, Mass.: *The number and distribution of incompatibility factors of Schizophyllum.*—All possible crossmatings within a sample of 98 homokaryotic strains obtained from widely separated locations revealed 85 distinct and interfertile *A* incompatibility factors and 51 distinct *B* factors. Assuming equal frequency of factors in each series, these results indicate about 360 different *A* factors and about 60 different *B* factors in the world-wide natural population. No *A* factor was recovered from more than two locations, whereas 15 of the 51 specific *B* factors were recovered from three or more locations. Duplication of neither factor was more frequent in a sample of 26 strains collected within 20 miles of Cambridge, Mass., than in the total sample. The distribution of specific *A* and *B* factors appears to be random in respect to geographical location, ecological habit, and accepted specific taxa.—The *A* factor involves a multilocus site, and 15 additional and 4 duplicate *A* factors have been obtained as recombinants in random pairings of *A* factors of the original sample. For example, two equally frequent recombinant classes among the progeny of the two *A* factors of a single dikaryotic stock collected in Cambridge, Mass., occurred as native factors in strains from Pennsylvania and Brazil, respectively. The frequency of duplication between recombinant and native *A* factors is indistinguishable from that among factors collected directly from nature. The extensive series of alternate *A* incompatibility factors thus appears to be maintained by frequent recombination between a number of distinct loci.

RASMUSEN, B. A., and C. STORMONT, University of California, Davis, California: *Blood groups in sheep. Serological reactions of erythrocytes of sheep which are analogous to those of the F-V system of cattle.*—The F-V system of cattle is a two-allele system with three phenotypes F, FV and V. Its elucidation came about by the observation that most anti-F reagents prepared from rabbit antiserum hemolysed red cells of type F more rapidly than those of type FV. Similarly, in sheep, it is found that two reagents, one called anti-X and the other anti-Z, prepared from rabbit antiserum differentiate three phenotypes X, X and Z, and here, as in the case of F and V, dosage reactions first called attention to the relationship. It was noted that the erythrocytes of sheep of type X were usually rapidly hemolysed by anti-X whereas those of type XZ were slowly hemolysed not only by anti-X but also by anti-Z. (Both rabbit complement, absorbed at 0° C to remove natural hemolysins for sheep, and guinea pig complement were used, and the dosage reactions were usually more pronounced with rabbit complement.) Although the serological results suggest that the genes controlling X and Z are alleles, the family data are as yet not sufficient to test the proposal adequately mainly because of the rarity of matings involving Z. Anti-X was first prepared by absorbing an anti-Karakul sheep serum and has since been duplicated in several antiserums against the red cells of sheep of mixed

European breeds. Anti-Z was first prepared from an anti-Springbok gazelle serum and has also been obtained from rabbit antiserums against domestic sheep.

RAY, DAVID T., Howard University, Washington, D. C.: *X-ray sensitivity of meiotic stages of Mormoniella eggs.*—Female wasps were treated with X-rays of different doses (1,000r—1,500r 2,000r—2,500r—3,000r), and set with host pupae of the fly *Sarcophaga bullata*. They were transferred to new host pupae at frequent intervals. Eggs laid at successive times after treatment were were treated at different meiotic (and even premeiotic) stages. Eggs laid within six hours after raying had been rayed during the first meiotic metaphase. Since the females were unmated they produced all haploid males parthenogenetically. Offspring per females as compared to controls furnished a rough estimate of the presence of lethals. Results seem to indicate that while the fecundity of treated females decreased with the increase of X-ray treatment, as expected, there was a drastic reduction in the number of offspring secured from eggs laid during the first six hours after treatments above 1500r. Reduction in the number of offspring as treatment increased from eggs laid in successive hours was not nearly as drastic. This indicates a higher sensitivity of the first meiotic metaphase to high doses of radiation with sensitivity decreasing in the earlier meiotic and premeiotic stages. A more accurate estimate is being made by egg counting. This was made possible by investigations during the summer of 1955, demonstrating rearing offspring from counted eggs (Damp chamber technique) hitherto not possible.

RICHTER, ALAN, University of Wisconsin, Madison, Wis.: *Complementary determinants of an Hfr phenotype in E. coli K-12.*—The mating types of *E. coli* previously described include F− (self-incompatible), F+ (moderately fertile) and Hfr (highly fertile). A number of Hfr strains have been isolated, all as mutants from F+ parents, and differing from F+ parents, and differing from F+ in the segregation of the Hfr quality as well as in their high fertility in crosses with F−. However, the relationship between the F+ determinant (which is readily transmitted by contact) and Hfr is not well understood.—Hfr_3 arose in a UV-treated F+ culture, simultaneously with a mutation, Mal_5^-, with which the Hfr quality appears to be closely linked. Hfr_3 cultures undergo a frequent, reversible change of state, from an extremely fertile, F-noninfective to an F-infective form. $Hfr_3 \times$ F− gives 80–90% F−, 10–20% F+. In addition, 0.01% of the progeny carry Mal_5^-, 98% of which show the Hfr_3 mating type. The remaining 2% of Mal_5^- F−, if exposed to F+ cells, are converted to the Hfr_3 type, as diagnosed by the specific segregation pattern in further crosses, and the oscillation between two states of fertility. This is in contrast to the majority of F− segregants which, like standard F−, become standard F+ on contact with F+ cells. The Hfr_3 mating phenotype therefore depends on two determinants, the F agent and Hfr_3^+. The four recognized phenotypes are: standard F−: Hfr_3^- F−; standard F+: Hfr_3^- F+; Hfr_3: Hfr_3^+ F+; segregant F− (potential Hfr_3): Hfr_3^+ F−.—Hfr_1 (CAVALLI and JINKS), Hfr_2 (HAYES) and Hfr_3 (these experiments)map as different loci on the bacterial chromosome. F has not been mapped as all the progeny of F+ × F are F+. The high incidence of F+ in the Hfr_3^+ class of segregants from $Hfr_3 \times$ F− suggests a special affinity of the F agent for the Hfr_3 locus. In Hfr_1 and Hfr_2, as previously speculated, the F agent may have become stably fixed to the corresponding *Hfr* loci.

RIZKI, M. T. M., Reed College, Portland, Oregon.: *Mitosis and somatic pairing in the imaginal discs of Drosophila larvae.*—The larvae of *Drosophila melanogaster* were fixed in Carnoy and Feulgen-stained whole mounts of various tissues were examined. Mitotic figures have been recorded in the imaginal discs, blood-forming organs, and the haemocytes. The chromosomes exhibit somatic pairing, and the two pairs of V-shaped chromosomes and one pair of rods in the female, and the XY in the male are easily identified. The IVth chromosomal pair is difficult to observe unless the positions of the chromosomes are favorable. The existence of somatic pairing in the chromosomes of the imaginal disc nuclei is of particular interest in view of STERN's genetic demonstration of somatic crossing over in Drosophila.

RUBIN, B. A., and E. B. WARD, Department of Public Health and Preventive Medicine, Baylor University College of Medicine, Houston 25, Texas: *A new genetic factor affecting tumor transplantation in mice.*—The immune reactions to antigens determined by the H2 system in mice are believed to be most important in affecting the compatibility of homotransplantation of normal tissues as well as of tumors. Other genetic factors affecting transplantation are known to exist because even in animals with identical H2 genetics, the rejection of homografts still occurs. Such a factor is evident in some recent studies in which it was found possible to cause the progressive growth of Gardiner Lymphosarcoma of C3H origin in the normally resistant Dba/2 and R III (to a lesser extent) mice by means of a pretreatment with carcinogens. These strains are of different H2 types from each other and from the C3H strain. Seven other strains of various H2 types failed to permit progressive growth with this type of treatment, although some improvement in temporary growth was usually evident. Of interest is the observation that some of the negative strains were initially able to grow this tumor far better than either of the reacting strains. When positive (reacting) strains are crossed with negative (nonreacting) strains, all of the progeny are non-reacting. Among the F_2 generation, 25% became susceptible to carcinogen enhancement. This classical recessive character is in sharp contrast to the invariable dominance of the H2 factors.—When the R III and Dba/2 are crossed, the F_1 progeny resemble the more poorly reacting strain, the R III. In view of this recessive nature of this effect, it is deduced that the same gene operates for both strains, but that some modifier exists in the R III, and perhaps more strongly (as several modifiers) in the very slightly reactive strains.

RUDKIN, G. T., and S. L. CORLETTE, Institute for Cancer Research and Lankenau Hospital Research Institute, Philadelphia, Pa.: *Nucleic acids and protein in puffs in giant chromosomes of Rhynchosciara angelae.*—PAVAN and BREWER (1952) have reported an apparent increase in Feulgen positive material associated with two of the puffs that occur in the salivary gland "B" chromosome of *Rhynchosciara angelae.* With their cooperation, we have undertaken the task of measuring the relative amounts of DNA, RNA, and protein in the same chromosome regions before, during, and after puffing, using standard cytochemical procedures. Salivary glands, fixed in 45 percent acetic acid in the cold, were squashed onto albuminized quartz slides. The preparations were digested successively with RNAase, DNAase and with hot 5 percent trichloroacetic acid. Photomicrographs suitable for measurement of total extinction by the methods of CASPERSSON were taken at 257 and 275 mμ in the ultraviolet after each treatment. Preliminary data show that the treatments are effective in removing ultraviolet absorbing materials ffom the chromosomes. From the tip of a "B" chromosome, RNAase removed 20 percent of the total extinction at 257 mμ, DNAase removed another 16 percent, while the final TCA treatment removed 37 percent, leaving 27 percent of the original total extinction on the slide. An absorption spectrum of this TCA extracted material showed a peak between 275 and 280 mμ. It appears that the two specific nucleases removed about half of the nucleic acids from the chromosome. These preliminary studies indicate that our analysis in progress will show whether or not there is a change in the nucleic acid or protein content of a chromosome region associated with puffing in that region. (Aided by grant C-1613 from the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service, and an institutional grant from the American Cancer Society.)

RUSSELL, LIANE BRAUCH, and L. WICKHAM, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee: *The incidence of disturbed fertility among male mice conceived at various intervals after irradiation of the mother.*—Strain (101 × C3H)F_1 females were mated at various intervals after receiving 400r X-rays and their sons were tested for fertility. Groups A–E consisted of 320 males conceived 1½–34½ days after irradiation; group O, of 80 contemporary sons of nonirradiated mothers. Results are based on about 7000 matings, made with 2454 females.—(1) Complete sterility occurred only among males conceived 13½ days or longer after irradiation. Its incidence in group C (13½–17½ days) is 6.2%; summed over groups A-E 1.9%. (2) Males giving infrequent litters due to many infertile matings were found only among sons of irradiated females (0.9%). (3) The incidence of males giving infrequent litters due to infrequent mating is equal among sons of irradiated

and control females (1.3% in each); among the former, however, there are another 2.8% with questionably reduced libido. (4) Slightly but consistently reduced littersizes characterized 1.3% of group O, 1.3% of groups A-E; while another 1.9% of groups A–E gave questionably reduced littersizes. Only one male, conceived 25½ days after irradiation, was clearly "semisterile," gave semisterile sons, and thus presumably carries a translocation. This result confirms that in mice, as in Drosophila, translocation induction in females is much less frequent than in males.—Summing all categories of disturbed fertility, the incidence among sons of irradiated mothers (5.4%; or 8.4%, if questionable cases are included) is higher, but not significantly so, than among sons of control mothers (2.5%). Group C has the highest incidence of disturbed fertility.

SAUL, GEORGE B. 2ND, Dartmouth College, Hanover, New Hampshire: *A new element at the R-locus in Mormoniella.*—Radiation studies have yielded two new mutants showing complementary allelism to known genes at the complex locus R in Mormoniella. The new mutants, having dark red (mahogany) eyes and designated *mh-846* and *mh-5101*, were crossed with *da-GF* and *oy-DR* at the O element of the R locus, with *vm-MK* and *st-DR* at the S element, and with *oy-NH* and *pe-333.5*, believed to overlap both elements. In all crosses, wild type F_1 females resulted and no recombinants were observed among F_2 males. It can be concluded that the new mutants represent a third R locus element for which the symbol M is proposed; that both genes are located at the same element is indicated by the fact that, when crossed, they give rise only to F_1 females with dark red eyes. Recombination of the M element genes with the non-R genes *pu* and *gl* is about 10.7% and 2.5% respectively, agreeing closely with data using other R locus genes.

SCHALET, A., Indiana University, Bloomington, Ind.: *Spontaneous mutations at specific X chromosome loci in Drosophila melanogaster.*—In a search for spontaneous visible mutations by MULLER's "Maxy" technique, approximately 150,000 daughters were examined for visible manifestations of 14 recessive markers provided in their maternal X chromosome, and suspected cases, presumably representing allelic mutations in their paternal X chromosome, were genetically analyzed. Simultaneously, approximately 100,000 sons were similarly examined, for mutation in their maternal X chromosome (not having these markers). The markers were yellow, prune, **white**, **echinus**, ruby, **carmine**, **cut**, singed, **raspberry**, dusky, **garnet**, **forked**, outstretched-small-eye, carnation. Bold face type indicates the 7 loci at which verified mutations were found in daughters. These arose singly except in case of cut, which arose once singly and in one cluster of 10 daughters. All mutants in daughters proved lethal except three: white (a "reddish" isoallele), garnet, forked. Sons showed two mutations of separate origin: a white and a cluster of 8 yellows. Since sons were not scorable for lethals or isoalleles, and poorly scorable for detrimentals and mutations of wing or eye conformation, these data do not indicate lower frequency for maternal chromosomes. It is true that tests for sex-linked lethals showed 0 in 613 maternal and 11 in 2421 paternal chromosomes, but there were 7 dominant Notch mutants among the 150,000 maternal X chromosomes of the daughters and only 3 among their paternal X chromosomes, thus evening the score. Relatively high mutation rate in early stages, giving clusters (see MULLER, 1946, a phenomenon suggesting interpretation of HILDRETH and CARSON's results), causes spontaneous frequency to be much higher, and sometimes with enormous error, when clusters are included. (This work was supported by a grant to H. J. MULLER and associates from the U. S. Atomic Energy Commission [Contract AT (11-1)-195]).

SEARLE, A. G. (introduced by CURT STERN), Department of Zoology, University of Malaya, Singapore: *Comparative genetics of some cat populations.*—Using the sex-linked yellow locus, large samples of London and Singapore domestic cat populations have been tested for conformity with the Hardy-Weinberg Law. In each city observed phenotype numbers agree well with those expected, suggesting strongly that mating is at random.—There are highly significant gene frequency differences between London and Singapore cats at several known loci, such as tabby and yellow. Incidence of the blotched tabby gene is 81.4% in London, but only 11.3% in Singapore; striped and Abyssinian (uniform) tabbies are much commoner in East Asia than in Europe. It seems likely that the blotched tabby mutant, unknown in wild relatives of the cat, arose first in Europe and has so far spread little in Asia.

The yellow gene is three times as common in Singapore as in London (31.5% against 10.7%) and even commoner in Japan according to KOMAI. Pure white cats are rare in all three centres, probably because they are at a selective disadvantage owing to associated deafness. 69.2% of Singapore cats show the kinky-tail anomaly, in which the tail is shortened and sharply bent, with fusions and distortions of caudal vertebrae. This abnormality is common throughout East Asia, but seems to be unknown in Europe except in Siamese breeds; it is not the same as the Manx condition.—Natural selection is clearly a much more important factor in the evolution of the cat than in that of most other domesticated animals.

SHIFRISS, O., Weizmann Institute, Rehovoth, Israel: *Sex expression in Ricinus.*—There are at least four forms of females in this normally monoecious genus. The genetically stable forms consist of (I) phenotypically persistent females, ($+S^u$), $+^{id}$, *f*, and (II) thermophotoperiod-induced sex-reversals, ($+S^u$), *id*, *f*. The genetically unstable forms consist of (III) graded series of female sex-reversals, (Su^{mf}), $+^{id}$, $+^f$, which give perpetually a spectrum of sex-reversals and monoecious individuals, the nuclear mutation from the state of femaleness to the state of monoecism being reversible and (IV) nearly stable category of nonreverted females, (Su^{m2}), *id*, $+^f$, which give over 95% nonreverted females, the mutation being primarily one directional. The genotypes are tentative. Symbol (Su^m stands for an unstable group of dominant nuclear factors which convert monoecious individuals, $+^f$, into females and determine the time of occurrence or nonoccurrence of phenotypic reversion to monoecism; *id* stands for a thermophotoperiod-requiring gene of unique behavior which brings about physiologically-induced staminate flowers in otherwise female differentiating tissue. Repeated tests have confirmed the existence of a paradox (Genetics 41: 265–280) in the breeding behavior of exceedingly late reverted females of form III. Employing a genetically stable tester, crossing these females before and after reversion shows clearly that phenotypic reversion from femaleness to monoecism is associated with a corresponding genetic change but selfing the very same racemes used in crossing does not allude to any genetic change. (Present address: Biology Department, Brookhaven National Laboratory, Upton, Long Island, New York.)

SIEGEL, A., W. GINOZA, and S. G. WILDMAN, University of California, Los Angeles: *Protection of tobacco mosaic virus infectious nucleic acid from ultraviolet light damage.*—Two strains of tobacco mosaic virus, U1 and U2, differ 5½ fold in sensitivity to inactivation of infectivity with ultraviolet light. The infectious nucleic acid preparations made from these strains are both identical in sensitivity to ultraviolet light. Furthermore, both nucleic acid preparations have the same sensitivity as the more sensitive U2 strain. These results were obtained by irradiating the preparations with a sterilamp (2537 A), in M/15 phosphate buffer, pH 6.9. Under these conditions both intact strains have approximately the same extinction coefficient and most of the energy absorbed is absorbed by the nucleic acid, in contrast to the protein which absorbs little at this wavelength. The conclusion is reached that the protein of the resistant U1 strain protects the nucleic acid from the damaging effects of ultraviolet light by a mechanism other than that of shielding. The protein of the U1 strain can protect only when it is hydrated. The sensitivity of the U1 strain becomes that of its free nucleic acid when the virus is dry during the irradiation. Drying does not affect the infectivity of the virus on subsequent rewetting and does not change the sensitivity of the U2 strain. These facts suggest that the protein of the U1 strain might be more hydrated in solution than that of the U2 strain. However, density gradient centrifugation in cesium chloride revealed the U1 strain to have a greater density under these conditions than the U2 strain.

SIEGEL, R. W., University of California, Los Angeles, Calif.: *An analysis of the transformation from immaturity to maturity in Paramecium aurelia.*—The life cycle of an exconjugant clone in *P. aurelia* may include a period of sexual immaturity during which cells multiply but cannot be induced to mate; maturity is reacquired in the course of further asexual reproduction. The persistance of each state, immaturity or maturity, through many asexual generations reflects a pattern of malleable cellular heredity controlled by unknown genetic factors. An investigation of the tranformation of immature to mature cells has revealed the following facts. First, the probability that a given cell will be mature

increases with its age (in number of fissions) since the origin of the exconjugant clone. Second, when stocks characterized by diverse periods of immaturity are crossed the progeny reveal a complex system of genic interactions (polygenes?); but it is clear that the genotype controls the duration of the period of immaturity. Finally, one basis for the variation in the duration of immaturity among isogenic clones has been discovered; if the first cells of an exconjugant clone to reach maturity are allowed to mate their progeny have long periods of immaturity; by contrast, if cells are mated only many fissions after the earliest onset of maturity their descendants will have markedly shorter periods of immaturity. (Supported by a grant from the National Science Foundation.)

SINGLETON, W. R., and A. L. CASPAR, The Blandy Experimental Farm, University of Virginia, Boyce, Va.,: *The Blandy radiation field for treating growing plants.*—A Cobalt-60 gamma radiation field has been established at the Blandy Experimental Farm. This is patterned somewhat after the first radiation field at the Brookhaven Laboratory with the modification that earth and concrete are used for shielding instead of distance as is used at the Brookhaven Laboratory. All direct radiation is shielded by the concrete and earthen embankments. Also, a concrete shield has been placed above the radiation source to shield the "sky-shine." The Cobalt source is approximately 200 curies in size and is sufficient to deliver enough radiation to induce changes in growing plants. The field will be used for producing both gametic and somatic mutations. The radiation area is a circle 30 feet in diameter and no plants will be grown in the field but all plants will be moved close to the source for a short time. In this way it is possible to obtain information on the most sensitive period in the life of a plant. The field will be used as much for obtaining information regarding the sensitivity of the various stages in the meiotic cycle and in premeiotic stages as it will be for plant breeding purposes. Visiting scientists are welcome to use this facility. Plants are currently being radiated for the Virginia Polytechnic Institute at Blacksburg, Va. (Supported by Contract AT-(40-1)-2071 between the University of Virginia and the Atomic Energy Commission.)

SIRE, MAJORIE W., and R. A. NILAN, State College of Washington, Pullman, Wash.: *The relation of oxygen to induced chromosome breakage in Crepis capillaris.*—Dry seeds of *Crepis capillaris* (2n = 6) were given X-ray dosages ranging from 1500r to 5000r. Pre- and postirradiation treatments of oxygen (100 lb. pressure) were applied to several samples. The seeds were then germinated and placed in .002 M 8-hydroxyquinoline when the root tip cells were undergoing their first mitotic divisions. Root tips were squashed in aceto-orcein. Dicentric chromosomes and rod and isodiametric fragments were recorded in the metaphase cells. The frequency of breaks in each chromosome arm was measured by the number of dicentrics involving that arm.—The long arm of C had relatively more breaks than were expected on the basis of length. Pre- and postirradiation treatments in oxygen did not alter the proportions of induced breaks in the six chromosome arms. However, these treatments did increase the frequencies of the X-ray induced dicentrics and fragments. Furthermore, the ratio of isodiametric fragments to rod fragments increased with the length of postirradiation storage in oxygen. (This work has been supported by a grant from the U. S. Atomic Energy Commission Contract AT(45-1)-353.)

SKAAR, P. D., Biological Laboratory, Cold Spring Harbor, N. Y.: *The tryptophan gene cluster in Escherichia coli B.*—All tryptophan requiring mutants of *E. coli* B. studied owe their requirements to closely linked genes, as in Salmonella (DEMEREC). Within this gene cluster, those mutants concerned with common steps in tryptophan synthesis show a contagious distribution. Where the ordering of genes in the cluster is clear, it corresponds to the ordering of synthetic steps. Posing a special problem are revertible mutants which lack two enzymatic specificities (YANOFSKY). These appear to be "point mutations" in contrast to those deletions which result in the loss of a number of enzymes plus the gaining of resistance to coliphage T1. Cistron assignations by means of OZEKI's abortive transduction technique are possible, and promise to clarify this situation. The principal results reported parallel those obtained in an independent study by LENNOX and YANOFSKY.

SMITH, BEN W., North Carolina State College, Raleigh, N. C.: *Chromosome behavior and sex ex-*

pression in induced polyploids of dioecious Rumex hastatulus.—*Rumex hastatulus* Baldw. is a dioecious, annual native to the Southeastern United States. Females are homogametic with eight chromosomes ($6a + XX$); males are heterogametic with nine chromosomes ($6a + Y_1XY_2$). At meiosis the sex chromosomes form a U-shaped trivalent which disjoins regularly so that the X or the two Y chromosomes are transmitted alternatively. Appropriate colchicine treatment of young seedlings produced tetraploid plants of both sexes (females $12a + XXXX$; males $12a + XXY_1Y_1Y_2Y_2$). Meiotic chromosome behavior in the autotetraploid males is remarkably regular because the chromosomes usually associate as in the diploid. The most frequent metaphase I configuration is $6_{II} + 2_{III}$ in which the autosomes pair instead of forming multivalents and the sex chromosomes associate in two Y_1XY_2 trivalents instead of forming pairs.—Representative autotetraploid females were pollinated by both diploid and autotetraploid males. Two triploids were obtained: a female with 12 chromosomes and a male with 13 chromosomes. If most of the functional gametes produced by the autotetraploids result from the diploid mode of pairing described, polyploid offspring should be produced with 16, 17, and 18 chromosomes in frequencies of 1:2:1. Approximately these frequencies of chromosome numbers did occur in the C_1 generation. The plants with 16 chromosomes were female; those with 17 chromosomes were male, but nearly half of them bore some intersexual flowers; plants with 18 chromosomes were male. There were three or four exceptional individuals in each class which require further study. Six aneuploid plants also appeared: one female with 15 chromosomes, four males with 19 chromosomes, and a 21-chromosome male which produced some intersexual flowers.

SMITH, STANLEY G., Forest Insect Laboratory, Sault Ste. Marie, Ontario: *Chromosomal evolution in Chilocorus stigma: an exception to "Robertson's law"*.—As a result of four centric fusions, *Chilocorus stigma* Say comprises an assemblage of chromosomal forms with diploid numbers ranging from 26 down to 18. In contravention of ROBERTSON'S "law" of numerical constancy, the number of chromosome arms (MATTHEY'S "N. F.") is concomitantly reduced from 52 to 36. This Robertsonian imablance is rendered possible, without resorting to pericentric inversion, by the dual nature of the unfused chromosome: basically, one arm is euchromatic (e) and is the site of chiasma formation; the other, of comparable size, is entirely heterochromatic (h) and is thereby precluded from chiasma formation. Dislocation of the arms through the centric regions of two such chromosomes (Ae.Ah and Be.Bh) can result in the formation of various telocentric and, after rejoining, metacentric elements. Of these, Ae.Be, being wholly euchromatic, forms a trivalent (Ah.Ae-Ae.Be-Be.Bh) in the heterozygote and a ring bivalent (Ae.Be-Be.Ae-) in the homozygote; Ah, Bh, and Ah.Bh, being constitutionally incapable of forming chiasmata, are either eliminated or, more rarely, float in the descendent population as supernumeraries. The limit of deviation from the "law", now standing at minus 16 arms, can be confidently set at minus 24: then, entirely lacking dispensable heterochromatin, the diploid number will have reached its ultimate low of 14.

SOKAL, ROBERT R., University of Kansas, Lawrence, Kansas: *Selection for pupation site differences in Drosophila*.—*D. melanogaster* when reared under controled conditions in six-dram shell vials containing corn meal-molasses-agar medium will pupate at various places along a gradient from the center of the medium surface to positions on the wall of the vial above the medium. Larvae were reared at a constant density of 10 per vial. Mass selection (on a combined individual-family merit basis) for differences in pupation site resulted in a peripherally pupating (PP) and a centrally pupating (CP) line. Response of the two lines was asymmetrical. The PP line responded immediately and strikingly with a plateau reached and maintained by generation 15, while the CP line reacted only gradually and not by very much. At generation 18 a maximum difference was attained when the PP line had 100% peripheral pupae while the CP line had only 6% such pupae. The differences declined somewhat during periods of relaxed selection but were quickly reestablished when selection was renewed.—Fertility and fecundity declined during selection, but recovered when the latter was relaxed. The two lines were examined for differences in the following characteristics: length of larval period, sex ratio, resistance of larvae to DDT in the medium and 14 morphological characters. Appreciable differences were found in all but the first two characters.—Crosses and chromosome assays have demonstrated that pupation site is a multifactorial character and have permitted estimates of

the contributions of the various chromosomes to the expression of the phenotype. Evidence is presented that selection in the two opposite directions has affected different genic systems. Eventual differences in pupation site are preceded by marked divergences in the feeding sites and activity patterns of the larvae of the two lines. (Studies aided by contract with Office of Naval Research—Nonr. 171(00).)

SONNEBORN, T. M., Indiana University, Bloomington, Ind.: *Early human foetal death rate in relation to paternal age.*—White foetal deaths up to the 20th week of gestation were examined separately in the first, second and third pregnancies for a relation to paternal age. (Data for 1954–55, New York City, obtained from the Department of Health, courtesy of C. L. ERHARDT.) When the maternal ages were grouped in 5 year age classes, the foetal death rates (expressed as percentage of live births) were consistently higher for fathers over 39 than for fathers less than 40. For example, in the third pregnancy, the older fathers had rates 20 to 40% higher than the younger fathers. However, this cannot be considered satisfactory evidence of a concentration of dominant lethals in older men. The foetal death rate changes markedly with maternal age *within* a 5 year age group and the older mothers within such a group have a disproportionate fraction of the older husbands. When maternal age classes are restricted to a single year, the number of foetal deaths from older (or younger) fathers becomes too small and irregularities in the relative rates appear. Data will have to be accumulated for 5 to 10 years to obtain decisive results. Thus far, the evidence is against a *steady* accumulation of dominant lethals with paternal age; but the possibility of specifically age-induced lethals, beginning at ages 40 to 45, is neither excluded nor demonstrated. (Supported by the Atomic Energy Commission and the American Cancer Society.)

SONNENBLICK, B. P., Newark College of Arts and Sciences, Rutgers University, Newark, N. J.: *A problem of human radiation genetics: Estimation of the dose delivered to the reproductive organs.*—It has become customary in recent reports to acknowledge generally or to particularize the fragmentary state of knowledge in several areas of genetics directly or indirectly applicable to the human instance. The exercise of listing for oneself those areas with severely limited information is a humbling one. But witness the plethora of views concerning problems of human radiation genetics, many by people of questionable experience but considerable assurance. One view, perhaps the only one, on which there can be widespread agreement among geneticists, and with other enlightened individuals is that current information, with its recognized strengths and weaknesses, dictates that indiscriminate usage of any mutagenic agent for man is ill-advised. Essentially this is what a handful of individuals has been attempting to disseminate in the years before the matter became so "popular".—Care is important less the argument be weakened, and it is somewhat disturbing to note recent comment concerning an "average" or even a "permissible" human gonadal radiation dose. How are the raw data for such estimates to be derived? For instance, with X-ray pelvimetric studies of late pregnancy, which we are elsewhere describing, and which are of particular genetic interest because of the dosages utilized as well the maternal and fetal gonadal involvement, do many appreciate that maternal ovaries are displaced by uterine enlargement during pregnancy to a position variable in each instance so that gonad dose estimations cannot be reliable? Fetal gonad estimations are unpredictable because of fetal positioning and movements. So too with other diagnostic procedures may several parameters variedly determine the radiation dose delivered to a critical site.—Quantitative estimates lend aura and an air of authority, but whether they are firmly or insubstantially based must be appreciated and expressed.

SPARROW, ARNOLD H., VIRGINIA POND, and RHODA C. SPARROW, Biology Department, Brookhaven National Laboratory, L. I., N. Y.: *Evidence for time of occurrence of the tertiary split in meiotic chromosomes of Trillium erectum.*—The tertiary split has been reported to occur at various stages in meiotic chromosomes in different organisms. Unequivocal visual evidence of the split is difficult to obtain because of the small diameter of the resultant strands and the complex coiling associated with meiotic chromosomes. Recent radiation experiments have, in our opinion, contributed some new information on the probable time of occurrence of the tertiary split in meiotic chromosomes of

Trillium erectum. This evidence is based on the assumption that certain breaks can give rise to bridges with "side arms" only if each chromosome of the bivalent is quadripartite. Analyses of the frequencies of such bridges were made in first anaphase cells which had been treated with 25r of X-rays at meiotic prophase and first metaphase stages The results indicate that Trillium pachytene chromosomes are not effectively quadripartite to irradiation but that diplotene, diakinesis and first metaphase chromosomes are. Analysis of microspore anaphase cells does not provide any evidence of a further subdivision (quaternary split) in late meiotic prophase or first metaphase chromosomes. The possibility that subchromatid breaks are a threshold phenomenon (such as "stickiness") has been investigated by irradiating anthers containing a mixture of cells at diakinesis and first metaphase with three dosages of X-rays (6.25, 12.5, and 18.75r). Preliminary data do not substantiate this suggestion. (Research carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.)

SPRAGUE, L. M., and C. STORMONT, University of California, Davis, California.: *The recognition of the soluble blood group substance Oc of cattle and its relationship to the blood group system J*.—The J system is one of 12 known blood group systems of cattle and has for years been regarded as a system comprised of two main phenotypes, namely J-positive and J-negative, with a variety of intergrades in the J-positive category. The J-positive phenotypes are recognized by means of natural isoantibodies found in the serum of most J-negative cattle. The J substance first makes its appearance as a soluble substance in the blood plasma and then some of it is acquired or absorbed by the erythrocytes. Its presence in the plasma in soluble form is readily detected by inhibition tests.—Another soluble blood group property detected only by inhibition tests employing so called cattle antisheep O antibodies has been found. This soluble substance has been designated Oc.—Although some of the Oc, like J, appears to be absorbed by the erythrocytes, they are neither agglutinated nor hemolysed by the action of anti-Oc in the presence of either guinea pig or rabbit complement.—With respect to the occurrence of J and Oc, four main phenotypes are recognized: J, JOc, Oc, and—(the absnece of J and Oc). Four main alleles have been postulated to account for the above phenotypic classes. Notably the characters JOc and Oc are postulated to be the products of alleles which act as recessives with respect to the occurrence of Oc.—The relationships which pertain between the phenotypic classes and the proposed genetic system as well as the origin of and cross reactions of natural anti-Oc are discussed.

SRB, ADRIAN M., Cornell University, Ithaca, New York: *Intra-colony selection of prototrophs occurring spontaneously in an adenine-requiring strain of yeast*.—When grown on appropriately supplemented 0.5% glucose agar medium, colonies of a red, adenine-requiring yeast present a homogeneous appearance, true to the color characteristic of the parent culture. On the same medium, but with 5% glucose, many of the red colonies show a striking overgrowth of white yeast that on isolation and subculture can be shown to be adenine-independent. Genetic analysis indicates that the prototrophic yeast results from back-mutation at the locus for adenine-requirement. Tests show that the medium with 0.5% glucose is at pH 5.0 while that with 5.0% glucose is at pH 4.5. At either sugar level, when the medium is buffered at pH 4.5, platings of adenine-requiring cells give red colonies with bursts of white, prototrophic yeast. At either sugar level, with the medium buffered to pH 5, or above, the bursts of prototrophic yeast fail to appear. The phenomenon of prototrophic bursts appears to be associated, therefore, with pH rather than sugar level *per se*. The nonappearance of bursts in red colonies grown at low sugar in unbuffered media or in media buffered to pH 5 is not due to the absence of prototrophic cells in these colonies, since such cells in fact can be shown to be present. In view of this, and since back-mutation tests do not show pH to affect reversion rate at the locus for adenine-requirement, it is supposed that pH level is associated with stringent conditions of selection for or against spontaneously occurring prototrophs.

STADLER, JANICE, and JOHN W. GOWEN, Iowa State College, Ames, Iowa: *Interaction of deleterious agents which may lengthen life span in female mice*.—Female life spans of a population of 10 distinct inbred strains of mice when subjected to radiation were 30% longer when exposed to 80 roentgens

whole body than when unexposed. The relation to radiation was progressive and peaked; 0r 391 days, 20r 445, 80r 508, 160r 488, 320r 478, 640r 370 and 960r 180. The male mates on the other hand showed a progressive detrimental effect of the X-rays. At first glance the results could be interpreted as an illustration of favorable effects of low quantity X-ray on the general functioning of life. That this is not so is shown by the effects on fertility. Females are subject to the hazards of the birth of offspring whereas males are not. There is a progressive decline in both numbers of pregnancies and numbers of offspring per pregnancy with radiation. At 0r, under the conditions of this experiment, the average life span females produced 5 litters, 20r 3, 80r only 0.5, 160r 0.4, 320r 0.2, 640r 0.04 and 960r 0.0. Numbers of young per litter were similarly reduced indicating an even greater progressive detrimental effect of the X-rays. These changes clarified the real effects of X-rays. At low doses the balance was in favor of the longer life spans for the mothers. By 320–640r the detrimental effects of the X-rays alone were sufficient to overbalance the favorable effects of not bearing young. Two agents, both of which were detrimental to duration of life, by their interaction, caused a spurious apparent increase in expected life span. (This work has received assistance from Contract No. AT(11-1)107 from the Atomic Energy Commission.)

STALKER, HARRISON D., Washington University, St. Louis, Missouri: *Sex-ratio in Drosophila paramelanica.*—The sex-ratio "SR" character in this species resembles that found in *D. pseudoobscura*. SR males produce 95–100% daughters, regardless of the females to which they are mated. The lack of sons does not depend on zygotic mortality, and is apparently associated with production of almost exclusively X-bearing sperm. SR males may also produce a few Y-bearing sperm resulting in fertile sons and a few nullo X-Y sperm which results in the production of sterile XO sons. The SR character has been found in populations from Mo., Ia., Minn., Wis. and Mich. In the St. Louis, Mo. strain the SR character depends on an X-linked factor or factors, male limited in expression, and apparently associated with a series of 5 short, X chromosome inversions. Extensive attempts to introduce the SR character from northern strains into the St. Louis genotype have so far failed. The data presently available indicate that the St. Louis strain carries one or more dominant suppressors of the northern-type SR. Thus not only do the northern and southern strains differ in the possession of such suppressors, but the primary SR genes must be different in the two, since the suppressor system of the southern strain does not suppress SR in that strain. Crossing data also indicate that neither permanent cytoplasmic differences nor Y chromosome differences between the northern and southern strains are the basis for the suppression effect. The study continues.

STINSON, HARRY T. JR., The Connecticut Agricultural Experiment Station, New Haven, Conn.: *Chlorophyll variegation in F_1 Oenothera hybrids.*—Interspecific crosses between certain geographical races of *O. parviflora* and *O. argillicola* give varying proportions of plants sectored green and yellow-green in F_1 when the former species serves as female parent. One such hybrid involves the circle 14, *parviflora* race *angustissima* as female parent and the 7-paired, alethal *argillicola* strain Douthat 4b as male parent. The F_1 chromosome configuration is a circle of 14, the complex-combination being alpha *parviflora*·^{h}*argillicola*. Self-pollination gives two cytological classes: 7-paired ^{h}*argillicola*·^{h}*argillicola* and circle 14 alpha *parviflora*·^{h}*argillicola*. Green and yellow-green branches arising from the sectors clearly breed differently on selfing and outcrossing. Both cytological classes derived from selfing green branches are fully green. When derived from yellow-green branches the alpha *parviflora*·^{h}*argillicola* plants are solid yellow-green, whereas the 7-paired *argillicola* segregates are solid green. The alteration in chloroplasts is not permanent, therefore, but persists only in the presence of the alpha *parviflora*·^{h}*argillicola* genotype. The ^{h}*argillicola*·^{h}*argillicola* segregates derived from green and yellow-green branches behave differently when crossed as female parents by the same male parents, but the segregates originating from yellow-green branches resemble closely the parental *argillicola* strain in breeding behavior. The two segregates yield similar results when used as male parents. This is taken to indicate a difference in some cytoplasmic component of the *argillicola* segregates from the two kinds of branches. These results, and those obtained from outcrosses of green and yellow-green branches, are compatible with the Renner concept of biparental chloroplast inheritance, followed by somatic segregation of maternal and paternal plastids.

STORMONT, C., W. J. MILLER, and Y. SUZUKI, University of California, Davis, California: *New phenogroups in the B system of bovine blood groups.*—During the past two years 5,000 cattle including Ayrshire, Brown Swiss, Guernsey, Holstein-Friesian, Jersey, Aberdeen-Angus and Herefords have been blood typed in this laboratory. The B system controlled by the largest allelic series analyzed in genetics, was extended by the recognition of 50 new phenogroups in these 5,000 animals. This brings the number of B alleles to 164. Many of the new phenogroups were found in the breeds least studied previously (Ayrshire, Aberdeen-Angus and Brown Swiss). When possible, absorptions of the reagents were performed with the new types of cells to characterize further the new phenogroups. Most of these previously unrecognized phenogroups are extremely rare, having frequencies less than 0.005.

SULLIVAN, ROBERT L., University of Kansas, Lawrence, Kansas: *Mutations in the housefly (Musca domestica L.).*—A search of the offspring of irradiated houseflies has resulted in the discovery of a number of mutations. Most of the mutations affect the wings. Breaks were found in the subcosta, the anterior and posterior cross veins, and the anterior and posterior regions of the fourth longitudinal vein. Additional veins have been found arising from the posterior cross vein and in the posterior regions of the fourth longitudinal vein. Other wing characters are: curly wings, vestigial (unexpanded) wings, and scalloping of the posterior margin and wing tip. Some of these are similar to mutants found by DR. MILANI of Pavia, Italy. One mutation affects the legs, causing a swelling, shortening and twisting of the femora. In all the above cases the penetrance and expressivity of the characters is highly variable. There is considerable serial and bilateral asymmetry in the expression of various characters. The character "shaven" causes a reduction of all bristles on the head, thorax and abdomen, leaving a short stubble. This character is lethal to flies prior to oviposition, lacking visible effect in heterozygous flies. Another character has been found which causes all male progenies. This character does not alter the size of progenies produced, indicating action prior to oviposition. All characters found to date appear to be recessive. (Supported by the Office of the Surgeon General, Department of the Army, contract number DA-49-007-MD-738.)

SUYAMA, Y., and V. W. WOODWARD, Kansas State College, Manhattan, Kansas: *Genetic analysis of pyrimidine mutants of Neurospora.*—The pyrimidine mutants of Neurospora studied to date fall into four genetic groups: Pyr-1, 2, 3, and 4. Pyr-1, 2, and 3 are located on the right arm of linkage group IV: the location of Pyr-4 has not been clarified. The order of linkage of the first three groups seems to correspond to the metabolic sequence of pyrimidine synthesis, and it should be noted that only the Pyr-3 locus has been characterized by more than two alleles.—An expansion of this general picture has been made possible through the procurement of 15 newly isolated pyrimidine mutants. On a physiological characterization, they are separable into four groups.—Crosses were made between the mutants and the original wild type, between the mutants and typ-4 (22 map units to the right of the centromere on linkage group IV), and between mutants in all possible combinations. The results obtained by linear dissection of asci indicate that all of the mutants (with the exception of one which has not been made to cross at all) fall in the interval between Pyr-1 and Pyr-3, and they are roughly separable into 3 groups rather than 2. But, they do not correspond to the original 4 groups deduced from phenotypic differences. The crosses between the mutants and typ-4 confirm the fact that at least 3, and probably 4, groups are concentrated in the interval frm Pyr-1 to Pyr-3. The new locus has been designated as Pyr-5. Crosses of mutants within these groups will be discussed. (This work was supported by a grant from the National Institutes of Health, RG-4561 [CS]. Contribution No. 588, Dept. Agron., Kansas Ag. Expt. Sta.)

TAYLOR, J. HERBERT, Department of Botany, Colombia University, New York: *Sister chromatid exchanges in tritium labeled chromosomes.*—Tritiated thymidine of high specific activity was used for labeling the DNA (deoxyribonucleic acid) of chromosomes in roots of *Bellevalia romana.* As in previous experiments (TAYLOR, WOODS and HUGES in Proc. Nat. Acad. Sci. US 43: 122–128, 1957) the chromosomes all contained tritium at the first anaphase after incorporation of the isotope. However, at the second division after labeling when one duplication in the absence of labeled precursors had occurred, only one of each two daughter chromosomes was labeled. This behavior shows that a chromosome is a duplex. Its two original strands are regularly separated during or after each duplication

so that an original and a new strand are passed on to each daughter chromosome. These two strands do not subdivide at subsequent duplications, but they do not remain intact because of sister chromatid exchanges. In Bellevalia the frequency of these exchanges was rather high. A type designated twin exchange can be recognized at the second c-metaphase following the labeling. In the presence of colchicine all of the chromosomes remain in one cell although chromosome duplication continues. In the tetraploid cells at the second c-metaphase after labeling, twin exchanges are recognized as exchanges at the same locus in two of the four homologous chromosomes. A high frequency of twins would be expected if all of the exchanges at the first interphase produced twins. This would happen only if the two strands of the chromosomal duplex are different in some way so that exchange and reunion of broken chromatids is restricted to the rejoining of like strands. Since the observed frequency can only be explained on the basis of this hypothesis, the two strands of the chromosomal duplex must be different in some sense. Calculation of the exchange frequency in the largest chromosome shows that there is an average of one exchange per chromosome at the first division cycle while only 0.21 exchanges occur at the second division cycle. Since the two cycles are the same except for the possible variations in the endogenous radiation from the tritium and the presence of the colchicine, one of these two factors must be affecting the frequency of sister chromatid exchanges.

VON BORSTEL, R. C., and MARY LOU PARDUE, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee: *Comparison of radiation-induced and genetically contrived dominant lethality in Habrobracon and Drosophila.*—In Habrobracon and Drosophila most of the deaths from irradiation of eggs and sperm occur early in embryonic development before the blastoderm is formed. The problem exists as to whether this early type of radiation-induced lethality can be attributed to gene imbalance. A solution to this problem may be reached by determining the time of death of inviable progeny of triploids or translocation heterozygotes. It has been found that aneuploid embryos from Habrobracon and Drosophila females die after blastoderm formation unlike the majority of radiation-induced dominant lethals. Corroborative evidence was obtained from examination of dead embryos from heterozygotes of two different Drosophila translocations and four different newly induced Habrobracon translocations; it was found in each instance that death is expressed after blastoderm formation. Since aneuploidy in neither Drosophila nor Habrobracon brings about death during early development that is most characteristic of radiation-induced lethality, it appears that radiation induces in large measure a type of dominant lethality that is not attributable to the loss of chromosomes or chromosome parts resulting from chromosome breakage.

WANG, CHI-WU, and T. O. PERRY, Forest Genetics, University of Florida, Gainesville, Florida: *Hereditary variation of the breaking of dormancy and the photoperiodic response in Betula.*—This study was based on a collection of birches made in 1953 throughout their natural range. Complete sets of the collection were established in Massachusetts, Florida, and Minnesota.—The results reported in this paper were obtained primarily from the collection growing under the natural short day length condition in Florida. Long day length condition was created by artificial illumination.—Observations made under uniform day length and temperate conditions in the last three years indicated that there are distinct differences among the plants from different geographic sources, both in their breaking of dormancy and in their photoperiodic responses.—Irrespective of photoperiods, the breaking of dormancy was accelerated by high temperature. The temperature for the breaking of dormancy was correlated to the temperature conditions of the geographic origin of the plants. Winter chilling was required for the normal breaking of dormancy for most of the plants. With the possible exception of a few, all the plants eventually grew after various lengths of delay.—The duration of the terminal growth was related to the photoperiod. Under uniform day length there was a distinct correlation between the cessation of terminal growth and the latitudinal origin of the plants. Hybrids of intraspecific crosses between plants of different geographic origins show characters that are intermediate to those of their parents.

WEINSTEIN, ALEXANDER, Biological Laboratories, Harvard University, Cambridge, Massachusetts: *Evidence that a short region can not contain more than one exchange.*—(1) Since there is at least one way of dividing every even number into two odd numbers, and every odd number above 1 into

three odd numbers, a multiple-crossover region of a chromatid is divisible, if nonrecombinant, into two recombinant regions; if recombinant, into three. Short regions (not exceeding 5 to 15 units, varying with location) cannot be so divided; hence they cannot contain more than one exchange.—(2) Replacement of single by multiple exchanges would, in general, produce too few tetratypes and attached-X homozygotes; replacement of noncrossovers by multiples would not sufficiently counteract this.—(3) In a short region of a tetrad, multiple crossing over producing four recombinant chromatids does not occur. Multiples producing four nonrecombinants are impossible because, not being directly detectable, they would, like sister-strand exchanges, decrease chances of detecting crossing over and hence would cause discrepancies; e.g., negative tetrad frequencies and too few tetratypes and attached-X homozygotes. If multiples producing two recombinant and two nonrecombinant chromatids in a short region are assumed, it is difficult to see what mechanism can prevent multiples producing four recombinants or four nonrecombinants. The discrepancies and difficulties are not avoided if a multiple is assumed to consist of one or more clusters of exchanges such as apparently occur in pseudoallelic crosses. They are avoided if a short region contains no multiples.—These and other considerations invalidate SHULT and LINDEGREN's criticisms of the crossing over theory of Drosophila workers. (The analysis summarized in the present abstract is part of a research supported by the Commonwealth Fund.)

WHITE, M. J. D., Dept. of Zoology, University of Missouri, Columbia, Missouri: *Genetic interaction of heterotic systems in grasshopper populations.*—In the Australian grasshopper *Moraba scurra* (of which over 8000 individuals from natural populations have been studied cytologically) two chromosome pairs ("CD" and "EF") are cytologically polymorphic for pericentric rearrangements (probably inversions). Three sequences ("Standard", "Blundell" and "Molonglo") are known for the CD chromosome and two ("Standard" and "Tidbinbilla") for the EF chromosome. The Molonglo sequence is only known from five localities and is always rare—but at two of these localities it has apparently displaced the Standard sequence. The Standard/Blundell CD and Standard/Tidbinbilla EF combinations give rise to heterotic effects on viability. But there is a complex genetic interaction between the two heterotic systems, so that the polymorphic equilibrium in the natural populations is due to both "within" and "between" chromosomal interactions. These relationships exist in two different geographic races of the species which differ in chromosome number, so that they are presumably of great antiquity. The Tidbinbilla sequence tends to be very rare or absent from colonies where the frequency of the Standard CD is high, due to a negative interaction. Thus, considering all colonies, there is a positive correlation between the frequencies of the Tidbinbilla EF and the Blundell CD sequences.

WHITING, P. W., The University of Pennsylvania, Philadelphia, Pa.: *R locus duplication and male diploidy in Mormoniella.*—Last year's Records, p. 666, reported a "mutation" duplicating the *R* locus in a male, no. 621.8, wild type and viable because of complementary allelism. Like his compound mother he was $+.st.l/oy.+.+$ carrying three mutant factors, scarlet and oyster eye color and a lethal, each recessive to its normal wild type alternative in the allelic pleiotropic gene. Two comparable "mutations" have occurred recently, but in each case the egg with the duplication was fertilized, resulting in a single wild type female among a large number of the expected red eyed sisters. Mutant female 726.8 was $+.vm/oy.+/da.st$ and mutant female 731.42 was $+.vm/oy.+/or.st$, each having received a double mutant gene (mutant in both *O* and *S* factors) from her father and the complementary alleles vermilion and oyster from her mother. Both females were of very low fecundity and both produced wild-type ($+.vm/oy.+$) as well as various mutant type sons. These wild-type sons proved highly fertile, siring many daughters which, like the two mutant females, were of very low fecundity, laying many eggs few of which hatched. Continued tests in succeeding generations, crossing the wild-type males to doubly recessive females, *oy.st* and *pe.st*, make it highly probable that these males are diploid, their daughters triploid. Unlike the near sterile biparental diploid males regularly produced from two-sex-allele crosses in Habrobracon, these highly fertile diploids (?) are impaternate. The data bear on the problem of sex determination.

WOODWARD, V. W., K. MUNKRES, and Y. SUYAMA, Kansas State College, Manhattan, Kansas: *Pyrimidine biosynthesis in Neurospora.*—Fifteen pyrimidine requiring mutants of Neurospora were isolated by the filtration and selective plating technique. One or more of the mutants fall into one of the following physiological groups: (a) growth is "normal" in the presence of 10 mM eq. of orotic acid, uracil, orotidine, uridine, and cytidine, but no growth occurs on any of the proposed precursors of orotic acid, e.g., oxalacetic acid, carbamyl aspartate, dihydro orotic acid. (b) same as (a) except no growth on orotic acid. (c) growth occurs only on orotidine, uridine, and cytidine; not on the free bases nor the precursors. (d) same as (c) except no growth on orotidine. (e) a secondary phenotype of the mutant in group (d) is a reduced ability (by $\frac{1}{3}$) to utilize cytidine and cytidylic acid.—These data do not demand the exclusion of orotic acid from the main pathway of pyrimidine biosynthesis; rather, they are compatible with the scheme proposed for pyrimidine synthesis in *E. coli*. However, an alternative hypothesis is that uracil is an intermediate between orotic acid and uridine.—The genetics of these mutants are of interest since all of the loci are located on the right arm of linkage group 4. It appears that there are at least five distinct groups of loci. (This work was supported by a grant from the National Institutes of Health, RG-4561 [CS]. Contribution No. 586, Dept. Agron., Kansas Ag. Expt. Sta., Manhattan.)

YERGANIAN, G., B. MACKAY, and H. GAGNON, The Children's Cancer Research Foundation and Harvard Medical School, Boston, Massachusetts: *The first ten generations of brother-sister matings of the Chinese hamster.*—Over 1000 litters have been raised since early 1952; of these, 497 were brother-sister matings (1 to 10 generations) involving 6 major agouti lines. Selection for docile, easily handled, early maturing, 4-day cyclic (estrus) colonizing females eliminated many sublines, in addition to the increasing sterility resulting from greater genic homozygosity. Spontaneous, chemical, and X-irradiation-induced tumors have demonstrated repeatedly the need for inbred strains to control histocompatibility or strain specificities for tissue transplantation. Prominent traits having multiple factor relationship are: (a) spotting—ventral patches of white hair; (b) "brittle bristle"—a sex-limited condition involving the guard hairs of the female only (BUY line), leading to varying degrees of alopecia; (c) unilateral cortical and medullary obliteration of the kidney, leading to hyperdiuresis and distention of the posterior portion of the vagina among the ORY and JFY sublines. The average litter size for random bred females is 5.5 and 4.5 for inbred lines. The "hen-pecking" sequence of dominance becomes fully expressed soon after the first female of a mixed litter reaches sexual maturity. Males of several VSY sublines mature earlier than their sisters, in contrast to the parental stock. Other VSY sublines have increased in body weight, whereas the JFY line, in general, has gradually experienced drastic reductions in vital statistics since the sixth generation. (This work was supported in part by NSF GRANT G-2829 and AEC Contract AT [30-1]-1753.)

ZALOKAR, M., Yale University, New Haven, Conn.: *Site of protein formation in the cell.*—A technique was developed for centrifuging living hyphae of Neurospora, by which the cell material could be sedimented inside the cell in the following order (starting at the centrifugal end): glycogen, microsomes, mitochondria, nuclei, cytoplasm, vacuoles, fat. Mycelium was fed C^{14}-proline for short periods immediately before centrifugation or during centrifugation. Radio autographs of fixed mycelium revealed β-tracks originating in the cell fractions which took up proline and presumably incorporated it into proteins. More activity was found in the nucleal fraction than in the microsomes. The significance of these results for the theory of gene action will be discussed.

ZAMENHOF, STEPHEN, ROSALIE DE GIOVANNI, and SHELDON GREER. Department of Biochemistry, Columbia University, New York, N. Y.: *Induced and spontaneous gene unstabilization.*—5-bromouracil and 5-bromouracil deoxyriboside induce mutants in *E. coli* in conditions in which 5-bromouracil was found to replace thymine in the deoxyribonucleic acid (DNA) of the living cells (J. Bacteriol. 71: 60, 1956). Some of the mutation rates are higher than 3×10^{-2} per cell per generation. The mutants are characterized by poor growth, but can be made to grow normally on addition of enrichments; this indicates the nutritional character of the mutants. Some of the mutants were unstable

for at least 180 generations. These mass changes are believed to be a result of a transient or permanent gene unstabilization caused by a transient disturbance in the chemical composition of DNA.—The spontaneous appearance and disappearance of gene unstability in a known unstable strain (ATCC #11887) was studied for comparison. It was found that the mutation from an unstable to a stable marker abolishes the high mutation rate to unstable genes in other loci, as determined by "differential counts" on full and basal media.—Several mechanisms for the preservation of unstable strains in mixed populations have been studied: it was found that the unstable strains may offer a selective advantage and become established. It is believed that these phenomena have evolutionary significance.

INDUCED CHANGES IN FEMALE GERM CELLS OF DROSOPHILA. II. OVIPOSITION RATE AND EGG MORTALITY IN RELATION TO INTENSITY AND DOSAGE OF X-RAYS APPLIED TO OOCYTES[1]

S. ABRAHAMSON[2] AND IRWIN H. HERSKOWITZ

Department of Zoology[3], Indiana University, Bloomington, Indiana

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AT the time gross chromosomal rearrangement frequency in Drosophila oocytes was found to increase with X-ray intensity, a significant decrease in the fecundity of the treated females was simultaneously noted (HERSKOWITZ and ABRAHAMSON 1956a). It seemed desirable to study this change in fecundity to learn to what degree it was based on a decrease in the number of eggs laid as opposed to an increase in the number which fail to hatch as larvae. The latter event, called egg mortality, may result from changes in the egg which are nongenetic, at least so far as the genes of the egg or zygote themselves are concerned, or genetic (dominant lethal mutations). In the experiments reported here oviposition rate and egg mortality were determined following treatment of oocytes with the same dosage of X-rays delivered at different intensities as well as after different dosages given in the same time interval. The present results with *D. melanogaster* on egg mortality and those obtained earlier by SONNENBLICK (1940) are discussed in relation to dominant lethal mutations induced in the female germ line. Some of the present work has been summarized elsewhere (ABRAHAMSON and HERSKOWITZ 1955).

MATERIAL AND METHODS

Wild type (Oregon-R) virgin females were collected and aged under excellent nutritional conditions for at least three days prior to treatment. The irradiations were given to motile, well aerated flies at 20 ma at a peak of 200 kv with 1 mm Al filtration. A Victoreen dosimeter was employed to determine the output of the X-ray machine just before the first irradiation of a series, and sometimes also between and/or after treatments. The delivery rate of the X-rays was varied either by changing the target distance or by stopping the machine periodically before the total dose was delivered or by both methods.

Simple, especially constructed, egg-laying units, modified from those used by MULLER (unpub.) and by TELFER (1954), were employed, each composed of 19 glass cylinders (25 mm in diameter and about 6 cm long) with one end covered by dacron netting held taut by cementing the netting to the wall of individual cylinders and with the cylinders held together in parallel in the shape of a hexagon by rubber bands. One female was placed in each cylinder with two males and the open end

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[2] Eigenmann Fellow in Zoology. Present address: Department of Genetics, University of Wisconsin, Madison, Wisconsin.

[3] Contribution No. 626.

plugged with cotton. The unit was then placed with the netting side down firmly on the surface of a large petri dish (15 cm in diameter) containing about one quarter of an inch thickness of preyeasted sugar-agar food. The females were then permitted to oviposit for about 12 hours, after which the egg-laying unit was removed, its netting brushed free of adhering eggs or food after about 20 minutes, and placed on a fresh nutrient-containing petri dish. One cylinder of each unit was labelled as was its position on the petri dish, so that from the imprint of the rims and netting of the cylinders on the medium it was possible to identify and keep a record of the eggs laid by individual females. As an aid in scoring the eggs from different cylinders a translucent plastic sheet, on which the outlines of the cylinders of a unit were traced, sectored, and numbered with India ink, was aligned under the petri dish.

Observations of the petri dishes were made, using transmitted light and a dissecting microscope, soon after the units were removed. At this time egg counts were made, and 36–48 hours later counts were again made, this time of unhatched eggs only. The petri dishes, whether carrying the laying units or not, were kept sealed from the outside in paper sacks except during scoring.

No attempt was made in these experiments to assure beforehand that the treated females carried lethal-free X chromosomes and no Y chromosome. As a means of compensation, at least 19 irradiated females were used per treatment.

It may be recalled that the most usual spontaneous mutation frequency of sex-linked lethals arising in either males or females is about .2 percent and that they tend to accumulate to only triple their frequency of origination, i.e. to .6 percent, in the chromosome that a female derives from her mother. Hence the usual chance of a female having such a lethal in one of her two X's is .8 percent, that is, only about one in 125 females carry a sex-linked lethal. Since only one quarter of the F_1 (one half those that were to produce males) of a lethal-bearing female are killed by the lethal, one in (4×125), that is, .2 percent, of the eggs laid by a large group of unrelated females collected at random will be killed by a sex-linked lethal, on the average. For counts of eggs derived from batches of 125 such females, the Poisson expectation is that in 37 percent of the batches no lethal-bearing female would have been present; in 26 percent one such female, resulting in .2 percent of egg mortality from this cause; in another 26 percent two such females, giving .4 percent mortality; in 8 percent three such females, giving .6 percent mortality; and in two percent four, giving .8 percent mortality. This gives .02 confidence limits at egg mortalities of .5 percent more and .15 percent less than the average, for counts from 125 unrelated mothers, and a correspondingly larger or smaller range for smaller or larger numbers of mothers, in inverse proportion to the square root of their number. The range would of course be greater for a given number of related mothers, since relationship would introduce the possibility of runs of lethals of common origin, and it would also be greater, by as much as ten-fold, if mutator genes were present in some or all of the individuals. (We are indebted to DR. H. J. MULLER for the clarifications presented in this paragraph.)

In all our experiments, the females were distributed randomly among the treated and control groups. Moreover, none of 103 control females ever gave egg mortalities which could be interpreted as resulting from any having carried a recessive sex-linked

lethal. Thus it is very unlikely that pre-existing lethals were frequent among the females. In a number of experiments performed later, which are not reported here, in which the female parents had been verified as being free of pre-existing lethals, it was found that if these experiments covered an aspect dealt with in the present experiments there was complete agreement in the results obtained.

Precaution was taken to avoid any non random effect of dehydration and starvation in flies exposed to treatment for different lengths of time. The longest treatment covered less than seven and one half hours. Flies which were given more concentrated treatments were irradiated either about midway in the period of time required for the diluter treatment, or were treated as two groups with one group receiving treatment toward the beginning and the other toward the end of the interval for the diluter treatment.

Experiments were performed at room temperature. Other procedural details or modifications of those already described are given with discussions of the results of specific experiments.

RESULTS AND DISCUSSION OF SPECIFIC EXPERIMENTS

In Experiment I 2000r were delivered either continuously at about 1266r/min (requiring only one hour 30 minutes for delivery) or at about 32r/min in eight equal parts with 24 minutes between the successive 8-minute long irradiations (taking three hours 52 minutes to deliver). After treatment females were placed in culture bottles for about 24 hours with an excess of Basc males, approximately 60 females and 120 males per bottle since the presence of numerous flies promotes mating. At the end of this time the males and females were placed into the cylinders of egg-laying units which were then transferred to new petri dishes in the manner and according to the schedule already described. The results are summarized in table 1.

In all experiments (as in Experiment I) in which males were present in the egg-laying units with the females, the first usable results for each individual female begin with the first one-half-day egg-laying period *following* the one in which collapsed eggs were first found (hence starting with an egg-laying interval after females had been inseminated). Accordingly, there are no data for the first 1 to 1.5 days after treatment for the females in Experiment I.

From 263 females in the diluted treatment test, 260 in the concentrated and 18 in the control, there were 11,902, 12,119, and 801 eggs oviposited, respectively, from days 1.5 through 3.5 after completion of the irradiation. Here, in the control, the difference due to recessive lethals, with a .01 confidence limit, would be 2.8 percent, that is, the control percent would have only one chance in 100 of being 2.8 percent too *high* if the mothers were unrelated. However, the data from these females proved that none carried a lethal from a previous generation. Hence this error is limited to treated females where individual egg counts would not surely tell when a pre-existing lethal was present. In these females the larger number used would greatly reduce the error. The similarity in number of eggs laid per female (45 for the diluted treatment, 47 for the concentrated and 44 for the control group) found in corresponding one-half-day intervals would seem to exclude difference in numbers of eggs laid as the basis for the large change in fecundity already noted in a similar experiment (HER-

TABLE 1

Experiment I. Egg mortalities obtained from irradiating oocytes with 2000r administered in concentrated form (1266r/min) or "diluted" (32r/min) in eight fractions separated by 24 minute intervals: total time three hours 52 minutes

Treatment		Days after irradiation				Total
		1.5–2	2–2.5	2.5–3	3–3.5	
Unirradiated (control)	No. eggs	124	194	351	132	801
	Percent unhatched	8.9	5.2	8.0	6.5	7.1
Concentrated	No. eggs	937	2443	5073	3666	12,119
	Percent unhatched	37.5	44.2	56.2	42.8	47.9
	Percent induced mortality (*A*)	32.7*	40.0	52.9	38.4	43.9
Diluted	No. eggs	753	2301	5244	3604	11,902
	Percent unhatched	19.4	25.9	38.5	27.0	30.2
	Percent induced mortality (*B*)	13.3	20.2	33.8	21.4	24.9
Difference in induced percent of mortality ($A - B$)		19.4	19.8	19.1	17.0	19.0
Intensity effect† (*C*)		22	25	29	22	25
C/*B*‡		1.65	1.24	0.86	1.05	1.00

* (37.5 − 7.1) divided by (100 − 7.1), × 100 = 32.7. This method of reckoning and those described below were suggested by DR. H. J. MULLER.

† Percent of survivors of diluted treatment killed by intense treatment = $100(A - B)/(100 - B)$.

‡ Intense effect relative to dilute effect.

SKOWITZ and ABRAHAMSON 1956a). Although there is no marked trend in percent of unhatched eggs in successive groups of eggs laid by control females in both the concentrated and diluted treatment tests the percentage of egg mortality rises successively in the first three groups of eggs and declines in the fourth. In each group of eggs, moreover, the results of the concentrated treatment show a higher incidence of induced egg mortality than do the diluted treatment results. These results, then, prove that a large component of egg mortality is dependent upon the additional X-ray intensity of the more intense series.

On the hypothesis that the fraction of induced egg mortality which shows this intensity effect is due to a multihit event, as in the production of half-translocations, it is of interest to inquire whether, as found for half-translocations (MULLER and HERSKOWITZ 1954; HERSKOWITZ 1954; HERSKOWITZ and ABRAHAMSON 1956a; PARKER 1954; ABRAHAMSON unpublished), the intensity effect is less (in relation to the total induced effect), in eggs oviposited later than it is in those laid earlier. If one supposes that the X-rayed oocytes laid in different periods subsequent to irradiation possess different susceptibilities to egg mortality that are due in part at least to some intensity-*independent* effect of the irradiation, then the greater this effect is the smaller will be the intensity-*dependent* difference observed. The last row of table 1 which gives the intensity effect relative to the total induced effect indicates that where the total effect is greatest, in the third half-day, the intensity effect is relatively the least. There is a suggestion that the intensity effect may become greater again after this period. Only one more half-day was studied in Experiment I, however, and one can hardly determine from this experiment whether the relative

intensity effect for egg mortality changes in the course of time as it does for half-translocations.

To obtain more definite information on these matters, Experiment II was performed. In this experiment 3000r were delivered either continuously at about 1266r/min (requiring only two hours and 30 minutes for delivery) or at 32r/min in 16 equal parts with ten minute intervals between successive six minute irradiations (taking four hours, six minutes to deliver). After irradiation, females and Basc males were placed directly in the egg-laying units and the transfer procedure as described was carried out. From 36 females in the diluted treatment and 38 in the concentrated there were 7631 and 8431 eggs oviposited, respectively, in the seven-day period of egg laying studied. The similarity in number of eggs laid per treated female, 212 and 220 respectively, supports a conclusion from Experiment I data that the difference in the way dosage was delivered did not appreciably influence the number of eggs oviposited. However, while the 2000r treatments in Experiment I did not result in any observable decrease in number of eggs laid as compared with the control, there seems to be a considerable decrease in number of eggs laid per female given 3000r treatment in Experiment II as compared with the 297 eggs per female in the control (5657 eggs laid by 19 females).

As was found in Experiment I the induced egg mortality in successive periods of egg laying tended first to rise and then to fall in both the concentrated and diluted treatment series but not in the control (table 2). Although in Experiment I the highest induced mortality was reached 2.5–3 days after irradiation and in Experiment II on 1.5–2 days after irradiation (a difference which could be due to factors varying in the two experiments, like the fact the parents came from different samples, the dose delivered was different, the handling of the females once irradiated differed, and especially the earlier start of intensive laying following irradiation) the concentrated and diluted series within an experiment reached this peak mortality at the same time. Also in Experiment II as in Experiment I the induced egg mortality in different periods of egg laying was always greater in the concentrated treatment group than it was in corresponding periods in the diluted treatment series. Moreover, this peak in both experiments was reached in the third half-day after intensive egg laying had begun.

Unlike Experiment I, in which the absolute difference between the concentrated and diluted treatments in induced percent of mortality in the third egg-laying period was not markedly different from earlier and later periods, in Experiment II (table 2) the third egg-laying period (1.5–2 days) shows a difference of only 7.8 percent whereas several of the periods before and after have values ranging from 18.0 to 28.2 percent. Since this seems to be correlated with the fact that the induced mortality in the diluted treatment is highest in that period, it is suggested that the results reflect a situation like that hypothesized in the preceding paragraph, i.e., that a relatively large portion of the additional killing at this period is not dependent on intensity. Thus, the intensity effect relative to the effect of the diluted treatment is lowest at the same time the diluted treatment gave most effect, namely, in the third period of egg laying in both Experiments I and II (last row of tables 1 and 2). Experiment II even suggests that in this period the intensity-dependent component *may* be ab-

TABLE 2

Experiment II. Egg mortalities obtained following irradiation of females with 3000r administered in concentrated form (1266r/min) or "diluted" (32r/min in 16 fractions separated by ten minute intervals: total time four hours six minutes)

Treatment		Days after irradiation												
		0.5–1	1–1.5	1.5–2	2–2.5	2.5–3	3–3.5	3.5–4	4–4.5	4.5–5	5–5.5	5.5–6	6–6.5	6.5–7	7–7.5
Unirradiated (control)	No. eggs	256	481	313	526	406	603	353	489	324	257	481	282	483	403
	Percent unhatched*	7.0	6.3	7.7	6.8	5.4	3.0	6.2	5.7	3.7	8.2	9.2	7.8	9.2	9.2
Concentrated	No. eggs	406	487	572	960	770	588	486	995	838	571	744	216	342	456
	Percent unhatched	62.5	67.5	82.3	70.2	55.5	47.0	43.4	37.3	36.2	31.8	30.1	28.2	32.4	29.6
	Percent induced mortality (*A*)	59.8	65.1	81.9	68.0	52.3	43.1	39.3	32.7	31.6	26.8	25.0	23.0	27.5	24.5
Diluted	No. eggs	58	134	351	783	738	720	503	715	648	404	770	518	699	590
	Percent unhatched	36.2	50.7	75.0	50.5	36.4	26.7	20.6	15.6	18.1	13.1	13.1	9.7	17.9	17.5
	Percent induced mortality (*B*)	31.6	47.1	73.2	46.9	31.8	21.4	14.8	9.4	12.2	6.8	6.8	3.1	11.9	11.5
Difference in induced percent of mortality ($A - B$)		28.2	18.0	7.8	21.1	20.5	21.7	24.5	23.3	19.5	20.0	18.2	19.9	15.6	13.0
Intensity effect† (C)		41	34	29	38	30	28	29	26	22	21	20	21	18	15
C/B‡		1.30	0.72	0.40	0.83	0.94	1.33	1.96	2.76	1.80	3.09	2.94	6.78	1.51	1.30

* Average value is 6.8 percent.
† $100\ (A - B)/(100 - B)$.
‡ Intense effect relative to dilute effect.

solutely, not merely relatively, smaller than in the periods before it and immediately after it (see next to last row of table 2) but this is not borne out by Experiment I.

It is clear, moreover, that in Experiment II less absolute intensity effect was found in the eggs laid in the last seven intervals studied than in the first seven (next to last line of table 2). The earliest period studied has the largest intensity effect while the last one studied has the smallest (and the first seven periods studied have greater intensity effects than the last seven periods in all cases). Thus just as for half-translocations the absolute intensity effect on egg mortality has been found to decline in later laid eggs. It is not evident, however, that the relative intensity-effect declines after the first four half-day periods; it seems instead to be higher.

In the case of half-translocations (HERSKOWITZ and ABRAHAMSON 1956a), the results indicated a higher relative intensity effect in eggs laid the first day after irradiation than in eggs laid the next three days. The report on half-translocations showed a much reduced absolute frequency of these changes following a continuous protracted as compared with a continuous concentrated treatment given at 1100r/min. Thus these results are similar to those obtained in the study of egg mortality. When, however, in another half-translocation experiment the protracted treatment was given in discontinuous irradiations with the total period of treatment, including

the intervals between exposures, equalling the time (about 30 minutes) required for the continuous protracted treatment in the former experiment, the paradoxical result was found that there was no detectable difference from the continuous concentrated treatment given at 1100r/min! Since the dosage used in the former half-translocation experiment (1000r) was only about one half that in the latter (2200r), the lack of detectable effect of dilution in the latter experiment may have been due either to the higher dose or the higher dose-rate used. However, in the latter experiment, when the intervals of nontreatment were extended there was a significant decrease in half-translocation rate in the most protracted as compared with the concentrated treatments. Whatever the causes of these results may turn out to be, it seemed reasonable to assume that if the intensity-dependent fraction of induced egg mortality results from multihit events, then by using the same intensity as in Experiment I's concentrated treatment (1266r/min) and both raising the dosage above 2000r and delivering the X-rays with varying periods of interruption, one might look for effects (within such an experiment and between it and Experiment I) similar to those found in the two half-translocation experiments mentioned.

Accordingly, in Experiment III (table 3) 2500r were delivered at 1266r/min in eight 15-second irradiations with nontreatment intervals of 0, 4, 16, or 32 minutes between, so that the time required for the respective treatments was two minutes, 30 minutes, one hour 54 minutes, and three hours 46 minutes. In this experiment the females were 14 days old when irradiated (about nine days older than the females

TABLE 3

Experiment III. Egg mortalities obtained after treatment with about 2500r delivered at 1266r/min in eight equal periods with the various time intervals indicated between successive irradiations

Minutes between irradiations	Total treatment time		Days after irradiation							Total
			1–1.5	1.5–2	2–2.5	2.5–3	3–3.5	3.5–4	4–4.5	
Unirradiated (control)	0	No. eggs	73	76	275	306	299	347	113	1489
		Percent unhatched (*A*)	4.1	3.9	12.7	13.7	12.7	4.6	9.7	9.9
0	2 min	No. eggs	203	166	483	500	533	550	321	2756
		A	54.1	37.2	68.0	69.8	57.2	36.6	28.3	52.5
		Percent induced mortality (*B*)	49.1	30.3	64.5	66.5	52.5	29.6	20.4	47.3 ± 0.95
4	30 min	No. eggs	216	125	417	456	576	663	301	2754
		A	52.7	39.2	57.4	68.2	58.8	37.7	28.6	50.5
		B	47.5	32.5	52.7	64.7	54.3	30.9	20.8	45.1 ± 0.95
		$4B - OB$	−1.6	+2.2	−11.8	−1.8	+1.8	−1.3	+0.4	−2.2
16	1 hr 54 min	No. eggs	124	100	344	347	410	408	131	1864
		A	17.7	20.0	56.1	63.4	46.3	30.2	28.2	43.2
		B	8.7	11.2	51.3	59.4	40.4	22.6	20.3	37.0 ± 1.1
		$16B - OB$	−40.4	−19.1	−13.2	−7.1	−12.1	−7.0	−0.1	−10.3
		Intensity effect* (*C*)	44	21	27	17	20	9	0	16
		$C/16B$	5.06	1.88	0.53	0.29	0.50	0.40	0	0.43
32	3 hr 46 min	No. eggs	118	58	236	280	391	402	223	1708
		A	39.8	22.4	62.0	61.0	43.6	26.4	29.6	42.2
		B	33.2	13.9	57.8	56.7	37.4	18.3	21.9	35.9 ± 1.2
		$32B - OB$	−15.9	−16.4	−6.7	−9.8	−15.1	−11.3	+1.5	−11.4
		Intensity effect† (*C*)	24	19	16	23	24	14	—	32
		$C/32B$	0.72	1.37	0.28	0.41	0.64	0.77	—	0.89

* $100(OB - 16B)/(100 - 16B)$.
† $100(OB - 32B)/(100 - 32B)$.

used in other experiments); they were mated to an excess of Oregon-R, wild type males for 24 hours, the males were then discarded and the females were placed in the egg-laying cylinders. For the control, and 0, 4, 16, and 32 minute intervals, 19, 38, 36, 28, and 19 females were tested and an average of 78, 72, 76, 67, and 90 eggs were laid per female, respectively. As in Experiment I, there does not seem to be any clear effect of the irradiation on the number of eggs laid per female.

The induced egg mortalities in different groups of eggs for the 32-minute intervals were lower than the corresponding groups for 0-minute intervals in seven of eight comparisons, and for the 16-minute intervals induced rates were lower than the corresponding 0-minute intervals in all eight comparisons. The overall induced egg mortalities for the 32-minute and the 16-minute intervals were also significantly different from the overall rate for the 0-minute intervals. The results for the 32-minute and 16-minute intervals were not significantly different from each other, although the overall intensity effect was greater in the former than the latter protracted treatment. Similar to the results with half-translocations is the fact that the intensity-dependent difference of 11.8 percent between the 0-minute and 32-minute intervals (in the period 1–3.5 days) was less than the 19 percent obtained in Experiment I, even though the duration of the protracted treatments was nearly the same in the two experiments and the latter involved a lower dosage of X-rays. The doses in Experiment III were delivered in the same way as in the half-translocation work (Experiment IV of HERSKOWITZ and ABRAHAMSON 1956a), the only difference being that the dosimeter reading was 1266r/min whereas it earlier had read 1100r/min when placed the same distance from the target with the machine's controls set the same way. In the comparable half-translocation experiment there was no detectable difference between the treatments with 0-minute intervals and 4-minute intervals. Similarly, for Experiment III, in three of eight comparable periods the induced egg mortality values for the 4-minute treatment group were higher than for the zero. Also, the overall rate for the four minute intervals is not significantly less than the zero minute. It may be concluded, then, that as with half-translocations, there is an intensity-dependent fraction of induced egg mortality which becomes smaller when irradiation treatment is both increased and delivered more intensely, even though the time for the total irradiation is kept the same, and which is even smaller when irradiation is given in several short intensive parts spaced over 30 minutes instead of being delivered intensively in two minutes.

Experiment IV (table 4) was similar in type to Experiment III. The dose was 3800r delivered at 1266r/min in twelve 15-second irradiations separated by 0, 4, 16, or 32 minutes (requiring three minutes, 47 minutes, two hours 59 minutes, and five hours 55 minutes, respectively, to complete the treatments). The number of females studied was 29, 35, 68, 47, respectively, and nine in the control, and the procedure for mating and egg laying were the same as in Experiment II. The number of eggs laid per female was, for the respective groups, 58, 113, 110, 122, and 101. In Experiment IV as in Experiment II, the average number of eggs laid by females receiving the most concentrated irradiation was less than the average produced by the control females. Following both the treatments given with 32-minute and 16-minute intervals each half-day egg mortality rate was lower than the corresponding rate

TABLE 4

Experiment IV. Egg mortalities obtained after treatment with 3800r delivered at 1266r/min in 12 equal periods with the various time intervals indicated between successive irradiations

Minutes between irradiations	Total treatment time		Days after irradiation								Total
			1–1.5	1.5–2	2–2.5	2.5–3	3–3.5	3.5–4	4–4.5	4.5–5	
Unirradiated (control)	0	No. eggs	72	130	91	130	95	133	97	130	912
		Percent unhatched (*A*)	6.9	17.7	4.4	11.5	5.3	11.3	5.2	10.0	9.3
0	3 min	No. eggs	71	245	102	178	330	283	195	266	1670
		A	64.8	70.7	82.3	86.5	73.0	56.2	50.7	54.0	65.7
		Percent induced mortality (*B*)	61.3	67.8	80.5	85.1	70.3	51.8	45.7	49.3	62.2 ± 1.2
4	47 min	No. eggs	220	512	389	719	622	513	313	685	3973
		A	52.3	75.0	91.8	75.4	58.5	47.6	39.9	39.1	60.4
		B	47.4	72.5	91.0	72.8	54.3	42.2	33.8	31.8	56.4 ± 0.8
		$4B - OB$	−13.9	+4.7	+10.5	−12.3	−16.0	−9.6	−11.9	−17.5	−5.8
16	2 hr 59 min	No. eggs	454	712	1062	1401	1328	848	663	1045	7513
		A	41.0	57.0	76.6	61.8	42.9	33.8	28.1	29.7	48.3
		B	34.9	52.6	74.3	57.9	37.1	27.0	20.7	22.5	43.0 ± 0.6
		$16B - OB$	−26.4	−15.2	−6.2	−27.2	−33.2	−24.8	−25.0	−26.8	−19.2
		Intensity effect* (*C*)	41	32	24	65	53	34	32	35	34
		$C/16B$	1.18	0.61	0.32	1.14	1.43	1.26	1.60	1.55	0.79
32	5 hr 55 min	No. eggs	470	582	813	924	859	595	482	1000	5725
		A	37.9	53.6	75.4	58.4	42.3	31.4	28.2	25.8	45.3
		B	31.6	48.9	72.9	54.2	36.4	24.4	20.8	18.2	39.7 ± 0.6
		$32B - OB$	−29.7	−18.9	−7.6	−30.9	−33.9	−27.4	−24.9	−31.1	−22.5
		Intensity effect† (*C*)	43	37	28	68	53	36	31	38	37
		$C/32B$	1.36	0.71	0.38	1.25	1.45	1.48	1.49	2.09	0.93

* $100(OB - 16B)/(100 - 16B)$.
† $100(OB - 32B)/(100 - 32B)$.

after treatment with 0-minute intervals (table 4). Although the 4-minute intervals had two of eight half-day values higher than those for the corresponding 0-minute intervals, nevertheless the overall rate for the former is significantly lower than for the latter ($P < .01$). Again, there was no significant difference between the results for 16-minute and 32-minute intervals, although the intensity effect of the latter was larger. While it is permissible to compare those results of Experiments III and I for which the duration of protracted treatments was almost identical, it is somewhat misleading to compare Experiment IV with either I or II in this manner, since the duration of the diluted treatments in IV is quite different from those in I and II.

The results for III and IV agree in that the amount of intensity-dependent egg mortality increases with the protraction of the treatments up to that of 16 minute intervals, and is comparatively small when the treatment extends over less than an hour. It should also be noted that, as in Experiment II, the two most protracted irradiations of both Experiments III and IV have peak periods of egg mortality with a somewhat smaller absolute intensity effect than do the periods just before and after the period of highest egg mortality, and they have (if the last period studied in III is discounted), as in Experiments I and II, by far the smallest intensity effect relative to the dilute effect of any of the periods studied.

TABLE 5

Experiment V. Egg mortalities obtained after treatment with 600r and 2400r delivered continuously at 1266r/min or interruptedly with 36 minutes between each irradiation at 32r/min in 18 one-minute and 18 four-minute irradiations, respectively

Treatment		Days after irradiation						Total
		1–1.5	1.5–2	2–2.5	2.5–3	3–3.5	3.5–4	
Unirradiated	No. eggs	195	180	696	370	814	464	2719
(control)	Percent unhatched	3.6	4.4	7.8	5.1	4.2	5.8	5.5
600r Intense (I)	No. eggs	539	675	1780	1502	2255	1407	8158
	Percent unhatched (A)	28.0	12.6	16.8	11.1	9.5	10.0	13.0
	Percent induced mortality (B)	23.5	7.5	12.0	5.9	4.2	4.8	7.9 ± 0.3
Dilute (D)	No. eggs	1004	765	2076	1610	2147	1548	9150
	A	9.9	10.5	14.6	10.4	7.9	8.4	10.3
	B	4.7	5.3	9.6	5.2	2.5	3.1	5.1 ± 0.2
	600 DB − 600 IB	−18.8	−2.2	−2.4	−0.7	−1.7	−1.7	−2.8
	Intensity effect* (C)	19.7	2.3	2.7	0.7	1.7	1.8	3.0
	C/600 DB	4.20	0.43	0.28	0.13	0.68	0.58	0.59
2400r Intense (I)	No. eggs	1018	564	2744	2141	3686	1596	11,749
	A	51.2	45.4	73.8	66.1	44.4	36.2	55.3
	B	48.4	42.2	72.3	64.3	41.2	32.5	52.7 ± 0.2
Dilute (D)	No. eggs	884	434	2561	1646	2463	1233	9221
	A	21.9	31.8	64.6	38.2	21.1	20.2	36.8
	B	17.5	28.0	62.5	34.6	16.5	15.6	33.2 ± 0.5
	2400 DB − 2400 IB	−30.9	−14.2	−9.8	−30.8	−24.7	−16.9	−19.5
	Intensity effect† (C)	38	20	25	47	30	20	29
	C/2400 DB	2.16	0.71	0.40	1.36	1.82	1.28	0.87

* 100(600 IB − 600 DB)/(100 − 600 DB).
† 100(2400 IB − 2400 DB)/(100 − 2400 DB).

If the part of egg mortality which is intensity-dependent depends on multihit events, then, as was previously found for half-translocations, one might expect this fraction to increase with dose at a rate faster than linearity. To test this, Experiment V (table 5) was performed in which 600r and 2400r were delivered. A continuous treatment at 1266r/min was given to furnish a lower and a higher dose given intensely and a discontinuous treatment with 36 minutes between each irradiation was given at 32r/min in 18 one-minute and 18 four-minute irradiations, respectively, in order to furnish a lower and a higher dose given slowly with interruptions. The lower intense dose took about 29.5 seconds to deliver, and the higher intense one minute 58 seconds; the lower dilute dose was delivered over a period of six hours 30 minutes, the higher dilute over seven hours 24 minutes. In both dilute and intense treatments the flies were X-rayed four times as long at the higher dose than they were at the lower. The intervals between irradiations in the dilute series are the same length of time for both doses. Consequently both groups of treated females had the same length of time for "repair" of "damage". The numbers of females studied were 38 in the control, 132 in 600r intense, 133 in 2400r intense, 133 in 600r dilute, and 124 in 2400r dilute treatment series. The numbers of eggs laid per female were 71, 62, 88, 69, and 74, respectively; these values do not suggest any significant effect of a dosage of 2400r on the number of eggs laid.

In every case in table 5 the egg mortality for a given dose was found to be lower in the dilute than in the intense treatment series. It is further to be noted that although the absolute intensity effect (next to last line of table 5) was not strikingly

low at the period of peak mortality which occurred the third half-day, the relative effect is at a decided minimum then. Similar observations are found in earlier experiments.

The overall intensity-dependent difference in induced egg mortality between the two 2400r treatments was 19.5 percent and between the two 600r treatments only 2.8 percent, the former being seven times the latter. On the basis of a linear increase with dose for the intensity-dependent fraction of egg mortality one would have expected a four-fold increase. Actually the increase might be expected to be less than four-fold if there is an intensity-independent fraction which at the higher dose is so large that it reduces the observed difference between the intense and dilute treatments by operating in the manner already described. The fact that the observed increase was seven-fold definitely places it above expectation on the linearity hypothesis. This is in agreement with the inference that much if not all of the intensity effect noted in Experiment V results from multiple X-ray "hits".

GENERAL DISCUSSION

The experiments performed furnish information concerning the effect of intensity and dosage of X-rays on rate of oviposition (table 6). In none of the tests in which irradiations of 2500r or less were delivered was there any sure change in the oviposition rate regardless of the intensity with which the dosage was delivered. The females laid between 84 percent and 124 percent as many eggs as did the control females. When 3000r and 3800r were given, however, three of the six groups of irradiated females gave only between 55 percent and 74 percent of the number of eggs produced by their respective control siblings, the other three groups having between 113

TABLE 6

Average number of eggs laid per female throughout the entire period studied and, as given in parentheses, in the period extending through the fourth day after irradiation (included to permit comparison with earlier work on half-translocations). C = Concentrated or intense treatments, D = Diluted or dispersed treatments

Experiment	Manner of treatment	Dose in r						
		0 (Control)	600	2000	2400	2500	3000	3800
I	C	44 (44)		47 (47)				
	D			45 (45)				
II	C	297 (154)					220 (112)	
	D						212 (91)	
III	$D_{0\ minutes}$ = C	78 (72)				72 (64)		
	$D_{4\ minutes}$					76 (68)		
	$D_{16\ minutes}$					67 (62)		
	$D_{32\ minutes}$					90 (78)		
IV	$D_{0\ minutes}$ = C	101 (75)						58 (42)
	$D_{4\ minutes}$							113 (85)
	$D_{16\ minutes}$							110 (86)
	$D_{32\ minutes}$							122 (90)
V	C	71 (71)	62 (62)		88 (88)			
	D		69 (69)		74 (74)			

percent and 120 percent the control oviposition rate. These data show that in *D. melanogaster* it is very likely that dosages lower than 2500r have a relatively small effect on oviposition and that dosages higher than 3000r decrease the number of eggs laid. Our results agree with those obtained by GOWEN and STADLER (1952) who found no effect on oviposition rate in *D. melanogaster* females treated with dosages up to 2500r but a decreased rate with higher dosages. Our results differ from those reported by ANNAN (1955) who found a decrease in oviposition rate of *D. robusta* females treated with a dose as low as 2500r. While differences between species or in dosimetry may account for this difference in results, it is likely that 2500r in *melanogaster* causes a decreased oviposition by an amount which is usually masked by the high variability in oviposition of control females.

Although there was a decrease in oviposition rate of irradiated as compared with unirradiated females in both Experiments II and IV, the results do not justify an alteration in the conclusions drawn from these experiments and those already reached from Experiments I and III which were similar in design but used lower dosages having no detectable effect on oviposition rate. Since from Experiment II it was concluded that the intensity effect was larger in eggs oviposited soon after treatment than those laid later, it became desirable to examine the data in terms of successive numbers of eggs oviposited rather than in terms of time of oviposition. This was done by rearranging the data from each female according to successive numbers of eggs oviposited as follows: The first group of eggs (labelled "1–15") contained all the data from the first egg-laying period throughout which the female was fertile up to but not including the period in which the 16th egg was laid, the second group (labelled "16–30") contained the data from the egg-laying period in which the 16th egg was laid up to but not including the one in which the 31st egg was laid, etc.; if any egg count contained so many eggs that it overlapped two groups, the data were placed in the later-oviposited group. The data for individual females used in Experiment II are summarized in table 7. (We are indebted to MISS SARAH CLEVENGER for executing these calculations.) Since in this experiment there were on the average 21 eggs laid per female per half-day in the seven days studied, the groups correspond approximately with the half-day periods, being about 25 percent smaller, so that the fourth group about corresponds with the third half-day.

We find no distinct rise in mortality after the first batch of eggs had been laid, either in the concentrated or dilute treatment series. Instead, all of the first three batches show a similarly high mortality and the mortality in succeeding batches in both series gradually falls. The absolute intensity effect (next to last line of table 7) follows a rather similar declining curve. As shown in tables 1–5, the relative intensity effect, taking the total effect at the dilute dose as the base of reference, does not show evidence of declining in the later batches but remains high in them (last line table 7). In the earlier batches the relative intensity effect is at a minimum, at the same stages as the total mortality is at a maximum, as shown in the other tables in which the data were arranged according to time. However, in table 7 where the data show that this mortality maximum is spread out over the first three batches rather than being concentrated in the third one the minimum of the relative intensity effect is similarly spread out and shows a slight tendency to be more extreme at only one

TABLE 7

Data from Experiment II, table 2, arranged in successive groups of eggs laid by individual females. See text for meaning of the groups and how these were determined

Treatment		Successive egg groups											
		1–15	16–30	31–60	61–90	91–120	121–150	151–180	181–210	211–240	241–270	271–300	>300
Unirradiated (control)	No. eggs	19	296	601	464	683	503	527	540	404	506	392	722
	Percent unhatched	10.3	9.5	5.7	6.7	4.0	7.2	5.1	6.5	8.7	8.5	5.2	4.6
Concentrated	No. eggs	204	536	1146	1129	1150	968	1221	767	629	372	154	155
	Percent unhatched	70.6	69.6	69.1	61.8	51.7	41.9	34.0	33.5	28.9	26.6	18.2	17.9
	Percent induced mortality (*A*)	69.0	67.0	67.0	59.0	48.2	37.7	29.2	28.7	23.7	21.2	12.2	11.9
Diluted	No. eggs	224	483	1012	979	1172	834	847	654	415	466	243	302
	Percent unhatched	52.2	54.2	48.5	32.6	23.5	15.7	14.5	13.9	12.5	14.4	6.6	9.5
	Percent induced mortality (*B*)	48.7	50.9	44.7	27.7	17.9	9.6	8.3	7.6	6.1	8.2	0.0	2.9
Difference in induced percent of mortality ($A - B$)		20.3	16.1	22.3	31.3	30.3	28.1	20.9	21.1	17.6	13.0	12.2	9.0
Intensity effect* (*C*)		40	33	40	43	37	31	23	23	19	14	12	9
C/B		0.82	0.65	0.91	1.55	2.06	3.23	2.77	3.02	3.12	1.71	>3.	3.10

* $100(A - B)/(100 - B)$.

point. This point lies in the second batch of eggs, corresponding in this case more nearly to the third half-day because of the very small number of eggs laid at first. It therefore becomes less certain that there is a sharp maximum of susceptibility to the production of intensity-independent damage to egg viability, lying at some stage separated from that of the first eggs laid. But it becomes more certain that the eggs of the earlier laid groups have, in general, a much higher susceptibility to damage of this kind than do those laid later, and that much of the excess of egg mortality at these more sensitive stages is caused by these intensity-independent events. It is the remainder—the intensity-dependent events—which correspond more nearly with the half-translocations that we have studied previously. That there is a maximum susceptibility to these events also in the earlier stages has been shown by the higher absolute intensity effect (next to last line in table 7) occurring in the earlier batches.

The present results and others mentioned for *D. melanogaster* make it clear that the investigations on the effect of X-ray delivery rate on the frequency of half-translocations (HERSKOWITZ and ABRAHAMSON 1956a) were uncomplicated by induced variations in oviposition rate, since the highest dose used in that work was 2200r. Since the study of the relation between half-translocation frequency and dosage (HERSKOWITZ 1954) used 4000r as the higher dose it is likely that the oviposition rate was lower for this treatment than for the lower dose treatment of 1000r. Since the frequency of half-translocations decreased in successive groups of eggs laid, if at the higher dose the same eggs were oviposited as at the lower one but at a slower rate, then the frequency observed at the higher dose may have tended to be biased as an

overestimate (see also HERSKOWITZ and ABRAHAMSON 1956a). However, other factors which tended to make the higher dose value an underestimate have been discussed (HERSKOWITZ 1954). These are in our opinion sufficient to counterbalance the effect just discussed and thus to allow the conclusions to be valid. Finally, it has been shown (ABRAHAMSON 1956) that for the doses 500r and 2000r (covering almost the same dose range as in the present Experiment V) in which the effect of radiation on egg deposition is negligible, half-translocations rose in frequency as the 3/2 power of the dose.

The experiments presented all agree that a large fraction of eggs which will survive diluted treatments with X-rays will perish if the same doage is given in a concentrated manner. This supports the view that much egg mortality is caused by multiple X-ray "hits". While there is no basis for concluding that *different* types of intensity-dependent events have been studied in the different experiments, there is also no proof that the *same* fraction was always studied, although economy of hypothesis requires this last view to be regarded as reasonable.

It had been the plan almost from the start of these investigations to see how much the results obtained in studies of the intensity-dependent fraction of egg mortality are in parallel with the results obtained with half-translocations. In all of the tests made there has been some similarity: 1. Intensity effects were obtained for both egg mortality and half-translocations when similar intensity changes were used over similar dose ranges. 2. The absolute intensity effect for both was greater in eggs laid the first four days after treatment than in eggs laid later. 3. The absolute intensity effect obtained for both events was greater when at the same time the dose was lowered and delivered less intensely, even though the time to complete the protracted irradiation was kept the same. 4. In experiments performed in the same way, in which the intervals between successive partial intensely-administered irradiations were varied but the total dose delivered remained constant, both types of effect varied according to the same pattern; namely, while 30-minute long treatments gave results which were not statistically different from results obtained after use of more concentrated treatments, the frequencies from two other still more protracted treatments, of the order of two to four hours or three to six hours, were significantly lower yet not significantly different from each other. 5. A similarity in the dependence of half-translocation frequency and the intensity-dependent fraction of egg mortality on X-ray dose was also found, in that both increased with dose at a rate greater than expected on a one-event hypothesis for their origin.

Because of these parallelisms it is concluded that almost all, if not all, of the intensity-dependent egg mortality represented results of multiple "hits" by X-rays. But however likely it may seem, there is no direct proof in the present results that any of the intensity-dependent egg mortality effect has a genetic basis. It might be assumed, without refutation being possible, that what has been studied is some nongenetic multihit event (such as one producing cytoplasmic damage) the behavior of which happens to parallel the multihit genetic event of half-translocation. It is nevertheless permissible to propose the hypothesis that part of the intensity effect studied in the present work has a genetic basis in multiple chromosome breaks, as does half-translocation. This is supported by the fact that there is very good reason

for believing, from the effect of ploidy on mortality (WHITING 1955) and the lower LD_{50} for X-rays when the nucleus rather than the cytosome is irradiated (ULRICH 1955), that genetic rather than cytoplasmic events are responsible for the great majority of egg deaths after oocytes are irradiated. Also supporting this opinion is SONNENBLICK's (1940) finding that in some Drosophila eggs which fail to develop after X-radiation there are chromosomal abnormalities which would be likely to cause death and WHITING's (1948, 1955) similar observations following irradiation of Habrobracon eggs at metaphase I.

Since it is extremely likely that egg mortality produced by X-raying sperm is largely due to genetic change in sperm-contributed chromosomes (MULLER 1940), it would seem reasonable to assume that such changes are to some extent capable of being induced by X-rays, with the same effect, in the chromosomes of oocytes. Such dominant lethal mutations would undoubtedly be composed not only of genetic changes requiring two or more but also of those produced by single "hits". Since, in the present studies, single-hit dominant lethal mutations would not be expected to show an intensity effect (unless additional special assumptions were made), deaths from this origin would be therefore included among the induced egg mortalities, especially with the more dilute treatments.

One can state that the intensity effect can kill 22.5 percent of all eggs laid and comprises 36 percent (22.5/62.2, in Experiment IV, table 4) of the induced egg mortality at a dose of 3800r delivered at 1266r/min. These are minimal values since even in the most diluted treatments some multihit events would occur.

Finally, it should be mentioned that if the intensity effects are due to multibreak events it would be desirable to obtain evidence concerning how much egg mortality could be due to eucentric half-translocations which killed because of aneuploidy, aneucentric full or half-translocations, or some multibreak event within a chromosome or tetrad. Experiments to test some of these possibilities have been completed and preliminary reports have been published (HERSKOWITZ and SCHALET 1956; HERSKOWITZ and ABRAHAMSON 1956b).

SUMMARY

A series of experiments similar to those performed to study the effect of X-ray intensity and dose on the frequency of half-translocations induced in oocytes was carried out with the purpose of studying egg mortality, as determined by the failure of eggs to hatch into larvae, and oviposition rate. As previously reported for *D. melanogaster*, it was found that doses up to 2500r produced no grossly observable reduction in the number of eggs laid, while higher doses did. The results also prove that there is an intensity-dependent fraction of induced egg mortality which can, under certain conditions of irradiation, result in the death of at least 22.5 percent of all eggs oviposited during the first five days after treatment. Parallelism was found between half-translocations and the intensity-dependent component of egg mortality in all comparisons of behavior which were made, comprising evidence that the latter is largely if not entirely the result of multiple X-ray-induced events. The hypothesis is advanced that part, at least, of the intensity-dependent fraction of egg mortality represents dominant lethal mutations which have their basis in two or more independently produced breaks in the maternal chromosomes.

In addition to the intensity-dependent fraction of egg mortality there is an intensity-independent (or relatively independent) fraction. This intensity-independent fraction is relatively very large in the eggs laid during the first two egg-laying days after irradiation (when the intensity-dependent fraction also is at a maximum but at a lower one) and it may have a rather sharp peak at a stage slightly separated from that of the eggs that were most mature at the time of irradiation.

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METHODS FOR CALCULATING AND EVALUATING A GENERAL SELECTION INDEX OBTAINED BY POOLING INFORMATION FROM TWO OR MORE EXPERIMENTS

W. D. HANSON AND HERBERT W. JOHNSON

Biometrical Services and Field Crops Research Branch, A. R. S., U. S. D. A., Beltsville, Maryland

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SELECTION indexes have been proposed in animal and plant breeding programs as a criterion for selection when certain quantitative characters are considered. Such characters present particular difficulty in selection programs since heritable variations are often masked by nonheritable variations. Investigations have demonstrated that a selection index based on a number of measurements could be used effectively to increase the probability of selecting desirable genotypes as compared to that if selection were practiced with an independent culling level for each measurement. The pertinent question is whether a selection index determined from a source of data can be used as a general index for a breeding program. The success of such a general index depends upon the sampling errors expected for the estimated weights of the index and the errors involved in the estimation of the genotype by environment interaction. The effects of genetic source sampled upon the expected genetic correlations between the factors involved in the index are of critical interest. Experimental data are necessary to adequately evaluate this problem; therefore, the approach considered in this paper is to present arguments for the combining of information from a series of experiments, to obtain a general index and to present basic relationships involved in the application of a selected set of phenotypic weights to specific sources of data.

Development of Relationships

The approach used here is essentially that outlined by SMITH (1936). Let ψ be the genetic worth of an individual and Y the corresponding phenotypic value. Then:

$$\psi = \sum_i a_i g_i, \text{ and } Y = \sum_i b_i p_i$$

where g_i is the genotypic value for a given character involved in a measurement, and a_i is the corresponding relative economic worth; p_i is the phenotypic value of the character, and b_i is any weight.

The variance of ψ is:

$$V_g = E[\psi - E\psi]^2 = \sum_i \sum_j a_i a_j g_{ij} = \sum_i a_i A_i, \text{ and} \qquad \text{(A)}$$

the variance of Y is:

$$V_p = E[Y - EY]^2 = \sum_i \sum_j b_i b_j p_{ij} = \sum_i b_i B_i, \qquad \text{(B)}$$

where g_{ij} and p_{ij} represent the genotypic and phenotypic variances or covariances, respectively, of the individual characters. The quantities, $A_i = \sum_j a_j g_{ij}$ and $B_i =$

$\sum_j b_j p_{ij}$, are identified primarily for calculation purposes. Similarly, the covariance between ψ and Y is:

$$W = E[Y - EY][\psi - E\psi] = \sum_i \sum_j b_i a_j g_{ij} = \sum_i b_i A_i. \tag{C}$$

The expected genetic advance (ΔG) for any set of b_j's is:

$$\Delta G = s\left[\frac{W}{\sqrt{V_p}}\right] \tag{D}$$

where s represents the selection differential in standard measure. The quantities, W, V_p and V_g, for any set of weights are identified in table 1. SMITH (1936) determined the values of b_j's which maximize genetic advance for a given source of data to be:

$$b_j = \sum_i c_{ij} A_i, \tag{E}$$

where the c_{ij}'s are the elements of the inverted phenotypic matrix. If one substitutes for b_j in (B) above, he obtains, $V_p = \sum_i b_i A_i = W$, and formula (D) for the expected genetic advance reduces to:

$$\Delta G = s\sqrt{W} = s\sqrt{\sum_i b_i A_i}. \tag{F}$$

If any other set of b_j's is used in the selection index, equalities E and F do not hold, and the expected genetic advance would be less than that expected if the weights calculated from (E) are used in the index.

The criterion for the establishment of an average selection index would be: when selection is to be based on a weighted index of two or more phenotypes, the weights will be determined such that the advance in genotypic worth will be a maximum with reference to all possible genotypes, for the characters under consideration, evaluated over all possible environments. This implies implicitly that the phenotype as deviation from $E(p_{ijk})$ must be considered as:

$$p_{ijk} = g_i + (Eg)_{ij} + \epsilon_{ijk}$$

where p_{ijk} and g_i have the interpretation previously noted, ϵ is the error within an environment (E) and $(Eg)_{ij}$ is the genotype environment interaction component.

In quantitative genetic research in plants, one cannot assume that $E(Eg)_{ij}^2 = 0$; rather, the set of genotypes arising from a cross or a group of crosses must be evaluated over a sample of environments to obtain an estimate of the variance associated with the environment by genotype interaction. Distinction is made between environments of location and environments of years. With genetic data evaluated over locations and years one would obtain a general selection index utilizing the method outlined by SMITH (1936) or HAZEL (1943). The model assumed for the analysis would be that genotypes, locations and years are random variables (ROBINSON 1951). It is obvious that the genotypes must be evaluated over both locations and years (or generations) before a reliable interpretation of the data can be made. Hence, the remainder of the theoretical discussion will be limited to data of this nature.

TABLE 1

Symbolic interpretation of W, V_g *and* V_p *for the* k*th source of data*

Genotypic variance-covariance matrix:[1]

Economic worth	Phenotypic weight	Economic worth			A_{ik}
		a_1	a_2	a_j	
a_1	b_1	g_{11k}	g_{12k}	g_{ijk}	$\sum_j a_j g_{ijk}$
.	.	.	.	.	.
a_i	b_i	g_{i1k}	g_{i2k}	g_{ijk}	$\sum_j a_j g_{ijk}$
.	.	.	.	.	.
.	.	.	.	.	.
.	.	.	.	.	.

Phenotypic variance-covariance matrix:[2]

Phenotypic weight	Phenotypic weight			B_{ik}
	b_1	b_2	b_j	
b_1	p_{11k}	p_{12k}	p_{1jk}	$\sum_j b_j p_{1jk}$
.	.	.	.	.
b_i	p_{i1k}	p_{i2k}	p_{ijk}	$\sum_j b_j p_{ijk}$
.	.	.	.	.
.	.	.	.	.
.	.	.	.	.

$$W_k = \sum_i b_i A_{ik} \qquad V_{pk} = \sum_i b_i B_{ik} \qquad V_{gk} = \sum_i a_i A_{ik}$$

[1] g_{ijk} = genotypic variance or covariance for the kth source.
[2] p_{ijk} = phenotypic variance or covariance for the kth source.

The purpose of combining two or more sources of data would be to minimize sampling errors, including the errors resulting from an inadequate estimation of the environment by genotype interaction, and to broaden the extent of sampling of genotypic or segregating populations. The problem is to obtain unbiased estimates of the genotypic, genotype by environment interaction and error variances. In experiments where sets of genotypes are evaluated under the same environments, the data would be analyzed as one experiment and would present no problem in combining. However, the more typical sources of data which could be considered for combining would involve sets of progeny lines evaluated at say 2–3 locations and 2–3 years. The problem is to combine the information from two or more such experiments. A simple (but approximate) procedure would be to average the estimates of the respective components, providing adequate degrees of freedom are available for each estimate. This is the same as pooling the respective genotypic matrixes and phenotypic matrixes where the phenotypic variances and covariances for the different experiments are expressed on a common base, that is, are expressed on the bases of equal replications, years, and locations. Genotypic and phenotypic variances and covariances may differ appreciably between experiments. An adequate criterion for combining appears

to be: The phenotypic weights (b_j's) will be selected such that the average genetic advance will be a maximum.

The average genetic advance for t sources of data is:

$$\Delta G = \frac{1}{k}\sum_{k=1}^{t}\Delta G_k = \frac{s}{k}\sum_{k=1}^{t}\frac{W_k}{\sqrt{V_{pk}}}$$

The maximum ΔG depends only upon the proportion included in the brackets. Maximizing the proportion in the brackets with respect to the b_i's yields simultaneous equations of the form:

$$b_i : \sum_j \sum_k \left(\frac{1}{V_{pk}}\right)^{1/2}\left(\frac{W_k}{V_{pk}}\right) b_j \mathbf{p}_{ijk} = \sum_j \sum_k \left(\frac{1}{V_{pk}}\right)^{1/2} a_i \mathbf{g}_{ijk}$$

or

$$\sum_j \sum_k b_j C_k \mathbf{p}_{ijk} = \sum_j \sum_k a_i D_k \mathbf{g}_{ijk}$$

where

$$C_k = \frac{W_k}{(V_{pk})^{3/2}} \quad \text{and} \quad D_k = \left(\frac{1}{V_{pk}}\right)^{1/2}. \tag{H}$$

Thus, the weights for combining the t phenotypic matrixes are proportional to the respective C_k's and for combining the t genotypic matrixes are proportional to the respective D_k's. The phenotypic weights for the selection index are calculated in the normal procedure from the pooled matrixes obtained using these weights, where the phenotypic matrixes are expressed on a common base.

An exact solution for the b_j's is impracticable; therefore, estimates of the average b_j's would be available if the phenotypic variance-covariance matrixes were simply pooled, as well as the genotypic variance-covariance matrixes, and the b_j's calculated. The values of the b_j's obtained can be used to estimate the respective C_k and D_k constants and to obtain a weighted pooled phenotypic and genotypic variance-covariance matrixes. One thus obtains a set of corrected average b_j's.

The arguments for the combining of data from two or more experiments have been outlined. The question to be considered next is the genetic progress expected in genotypic worth or in the individual characters if phenotypic weights other than those which maximize genetic advance for the source of data are considered. Such a set of phenotypic weights could be based, for example, on the economic worth of the characters in the index, on data obtained from a genetic population with characteristics distinct from the genetic source under consideration, or on some accepted or assumed general index. SMITH (1936) simplified formula (D) for genetic advance for the specific case which maximizes genetic advance for the source. For any other set of b_j's the expected genetic advance for a selected group is:

$$\Delta G' = \frac{sW}{\sqrt{V_p}} = s\frac{\sum_i b'_i A_i}{\sqrt{\sum_i b'_i B'_i}},$$

where the primes are used to identify any selected set of b_i's as compared to that set which yields maximum expected genetic advance for the selected data ($\Delta G \geq \Delta G'$).

The expected correlation (r) between the index of phenotypic values when the specific index is used and when the general index is used is:

$$r = \frac{\sum_i b'_i B_i}{\sqrt{\sum_i b_i B_i \sum_i b'_i B'_i}}. \tag{I}$$

For this specific source,

$$\sum_j b_j p_{ij} = \sum_j a_j g_{ij},$$

hence,

$$\sum_i b'_i B_i = \sum_i b'_i A_i \quad \text{and} \quad \sum_i b_i B_i = \sum_i b_i A_i.$$

The constant $K = \frac{V_p}{W}$ noted by SMITH (1936) is unity for the selected case. The expected correlation reduces to:

$$r = \frac{\sum_i b'_i A_i}{\sqrt{\sum_i b_i A_i \sum_i b'_i B'_i}} = \frac{\Delta G'}{\Delta G}. \tag{J}$$

The ratio of the expected genetic advance for any selected set of b_j's to the maximum genetic advance expected through the use of an index is essentially the expected correlation between the respective indexes of phenotypic values.

The expected genetic advance (ΔG) which has been determined is the expected advance in the genotypic worth (ψ) for the selected group. The expected genetic changes for the individual characters involved in the index can be calculated. The symbol ${}_j\Delta G_{a,b,\cdots}$ will be defined as the expected genetic change for the jth character (in the units of the character) when characters $i = a, b, \cdots$ are used to construct the index. For any selected set of b_i's:

$${}_j\Delta G'_{a,b,\cdots} = s \frac{\sum_{i=a,b\cdots} b'_i g_{ij}}{\sqrt{\sum_{i=j=a,b,\cdots} \sum b'_i b'_j p_{ij}}}. \tag{K}$$

For example, the expected genetic change in X_1 when the index is based on X_1, X_2 and X_3 is:

$${}_1\Delta G'_{123} = s \frac{\sum_{i=1}^{3} b'_i g_{i1}}{\sqrt{\sum_{i=1}^{3} \sum_{j=1}^{3} b'_i b'_j p_{ij}}}$$

which reduces to:

$${}_1\Delta G_{123} = s \sqrt{\sum_{i=1}^{3} b_i g_{i1}}$$

if $b_i = \sum_j^3 c_{ij} g_{ij}$ is considered. These weights yield maximum expected genetic advance for X_1. The expected genetic advance in X_1 if the selection index is based on X_2 and X_3 is:

$$_1\Delta G_{23} = s \frac{\sum_{i=2}^{3} b'_i g_{i1}}{\sqrt{\sum_{i=2}^{3}\sum_{j=2}^{3} b'_i b'_j p_{ij}}}$$

which reduces to:

$$_1\Delta G_{23} = s \sqrt{\sum_{i=2}^{3} b_i g_{i1}}$$

if $b_i = \sum_{i=2}^{3} c_{ij} g_{i1}$ is substituted in the formula. The c_{ij}'s in this case are the elements of the matrix resulting from the inversion of:

$$\begin{matrix} p_{22} & p_{23} \\ p_{32} & p_{22}. \end{matrix}$$

The relationship for expected correlation between the indexes of the phenotypic values holds. For example, $_1r_{123} = \frac{_1\Delta G'_{123}}{_1\Delta G_{123}}$, is the expected correlation between the indexes of phenotypic values for X_1 when the two respective indexes are used.

NUMERICAL EXAMPLE

The data for this example have been obtained from JOHNSON, *et al.* (1956). In this reference selection indexes based on different criteria have been evaluated for two different segregating populations (experiments) of soybeans. The populations have also been described in JOHNSON *et al.* (1955). The lines were evaluated in the F_4 generation at two or three locations in one year. Although the mean score ratings for lodging were very similar for the two populations, the response to lodging was distinct, population one being characterized by loss of dominance of the terminal bud and population two being characterized by maintenance of dominance of the terminal bud in the lodged plants. The data in grams per plot involving yield of oil (X_1), yield of protein (X_2), and yield of seed (X_3) were selected for this example. The economic worth equivalents of $a_1 = 1$, $a_2 = .6$, and $a_3 = 0$ for X_1, X_2 and X_3, respectively, were also selected for this example. The phenotypic and genotypic variance-covariance matrixes are given in table 2. The phenotypic weights which maximize expected genetic advance in the specific populations and the phenotypic weights computed from the pooled variance and covariances also are included in the table. The calculations are identical to those outlined by SMITH.

The variances of the phenotypic value and the genotypic worth and the covariance between the phenotypic value and the genotypic worth were calculated as outlined in table 1 using the b values estimated in table 2. An example of computational

procedures necessary for calculating W_1 for segregating Population I is outlined in table 3. The respective variances and covariances were estimated as follows:

	Segregating population	
	I	II
V_{pk}	177.29	154.68
W_k	174.29	157.68

The weights for pooling the phenotypic matrixes and the genotypic matrixes were calculated by formula (H) and are as follows:

	Segregating population	
	I	II
C_k	.07383	.08196
C_k'	1.0000	1.1101
D_k	.07510	.08040
D_k'	1.0172	1.0890

Since the weights are relative, the weights can be expressed as a proportion of .07383.

The two populations are not appreciably different with respect to the variances and covariances involving genotypic worth and phenotypic value. The weights for combining the respective matrixes are essentially unity. The phenotypic variance-covariance matrixes and the genotypic variance-covariance matrixes were pooled proportionately to the weights C_k and D_k. The phenotypic weights were again calculated in the normal procedure yielding:

Weights	Phenotype		
	X_1	X_2	X_3
Calculated	.8290	.1073	−.0507
Relative	1.0000	.1294	−.0612
$b_i/\sqrt{cjj}$	5.01	1.11	−.77

The corrected relative phenotypic weights do not differ appreciably from the estimates obtained by simply pooling the genotypic matrixes and the phenotypic matrixes. It also should be noted that the index is determined principally by yield of oil (X_1).

The maximum genetic advance for a population can be computed directly from $s\sqrt{\Sigma b_i \bar{A}_i}$. As an example, the calculations necessary to compute the genetic advance if the weighted index were used are given in table 4. The calculations necessary to compute r based on relationships (I) or (J) are also indicated.

The relative phenotypic weights based on the specific indexes for each source, on the average index and on economic worth are presented in table 5. The genetic advances expected together with the expected correlation of phenotypic values for each of these indexes are given in table 6. The selection indexes are similar in that

TABLE 2

Phenotypic and genotypic variance-covariance matrixes obtained from two segregating populations of soybeans

		Segregating population					
		I			II		
		X_1	X_2	X_3	X_1	X_2	X_3
Phenotypic[1]	X_1	287.455	477.40	1,266.170	247.89	442.07	1,136.425
	X_2	477.40	934.95	2,303.13	442.07	1,219.54	2,591.68
	X_3	1,266.170	2,303.13	5.951.19	1,136.425	2,591.68	6,075.24
Genotypic	X_1	128.730	160.607	492.532	112.13	139.378	425.19
	X_2	160.607	254.56	707.74	139.378	482.058	935.98
	X_3	492.532	707.74	2,103.24	425.19	935.98	2,245.03
Specific phenotypic weights		$b_1 = 1.2433$ $b_2 = -.5942$ $b_3 = .1196$			$b_1 = .7283$ $b_2 = .3401$ $b_3 = -.1189$		
Pooled phenotypic weights		$b_1 = .8335$ 1.0000	$b_2 = .0968$ .1161	$b_3 = -.0476$ $-.0571$			

[1] Phenotypic variances and covariances were constructed on the assumption that genotypes were to be evaluated over one year and two locations with two replications per location.

TABLE 3

Computational procedures for the calculation of W *for Population I utilizing the pooled phenotypic weights*

Phenotypic weight	Economic worth			A_i
	1	.6	0	
.8335	128.730	160.607	492.532	225.09
.0968	160.607	254.56	707.74	313.34
$-.0476$	492.532	707.74	2,103.24	917.18

$$W = \sum_i b_i A_i = 174.29$$

the weights associated with X_1 are the largest of the three phenotypic weights and are positive. There are differences in the contribution of X_2 and X_3 to the selection indexes. The genetic advances, however, are quite similar for a population when the different indexes were used, the exception being the index from Population I applied to Population II. Likewise the correlations of phenotypic values are surprisingly close to 1.

Consideration has been given to the expected advance in genotypic worth where genotypic worth has been based on the relative economic worth of the three characters involved. The analysis of the change occurring with each character should be made. It should be noted from formula (K) that ${}_1\Delta G_1$ is a special case of ${}_1\Delta G_{123}$ where

TABLE 4

Calculation required for the estimation of genetic advance and correlation of phenotypic values for population I

Genotypic variance-covariance matrix:

b	b'	a_i			A_i
		1	.6	0	
1.2433	.8290	128.73	160.607	492.53	225.09
−.5942	.1073	160.607	254.56	707.74	313.34
.1196	−.0507	492.532	707.74	2,103.24	917.18

Phenotypic variance-covariance matrix:

b	b'	b 1.2433	−.5942	.1196	B_i'	B_i
		b' .8290	.1073	−.0507		
1.2433	.8290	287.455	477.40	1,266.17	255.33	225.16
−.5942	.1073	477.40	934.95	2,303.13	379.32	313.46
.1196	−.0507	1,266.17	2,303.13	5,951.19	995.06	917.47

$$\sum_i b_i' A_i = 173.72 \qquad \sum_i b_i A_i = 203.36$$

$$\sum_i b_i' B_i = \sum_i b_i B_i' = 173.78 \qquad \sum_i b_i' B_i' = 177.05$$

$$\Delta G = s \frac{203.36}{\sqrt{203.41}} = s(14.26) \qquad \sum_i b_i B_i = 203.41$$

$$\Delta G' = s \frac{173.72}{\sqrt{177.05}} = s(13.06)$$

$$r = \frac{173\,78}{\sqrt{(203.41)(177.05)}} = .916 \qquad r = \frac{13.06}{14.26} = .916$$

TABLE 5

Summary of the relative phenotypic weights based on the specific indexes for each population, on the average index and on an index of economic worth

Source	Phenotype		
	X_1	X_2	X_3
Population I	1.0000	−.4779	.0962
Population II	1.0000	.4670	−.1633
Weighted average	1.0000	.1294	−.0612
Economic worth	1.0000	.6000	.0000

the weights are taken to be: $b_1 = 1$, $b_2 = 0$ and $b_3 = 0$. Hence, ${}_1\Delta G_1 \leq {}_1\Delta G_{123}$ and the expected correlation between the yield (X_1) of oil and the index of phenotypic values is $\frac{{}_1\Delta G_1}{{}_1\Delta G_{123}}$ where ${}_1\Delta G_{123}$ is the maximum genetic advance expected for X_1.

The weights which yield maximum expected genetic advance (${}_j\Delta G_{123}$) for each character within each population are given in table 7. The lack of similarity between

TABLE 6

Summary of genetic advances expected together with the correlation of phenotypic values when different selection indexes are used

Source phenotype weights	Population I		Population II	
	$\Delta G/s$	Correlation	$\Delta G/s$	Correlation
Population I	14.26	1.00	7.55	.53
Population II	12.70	.89	13.08	1.00
Weighted average	13.06	.92	12.71	.97
Economic worth	11.94	.83	12.98	.99

TABLE 7

Phenotypic weights yielding maximum expected genetic advance for each character within Populations I and II

Population	b_i	Character		
		Oil (X_1)	Protein (X_2)	Yield (X_3)
I	b_1	1.05867	.30784	.99475
	b_2	−.38167	−.35423	−2.14698
	b_3	.00522	.19051	.97265
II	b_1	.87309	−.24193	−.15114
	b_2	−.04128	.63575	−.24750
	b_3	−.07572	−.07188	.50341

the corresponding indexes for Population I and Population II is striking. The expected change for a character within a population when one of the four indexes given in table 5 is used as a criterion for selection can be obtained relatively easy from the calculations as outlined in table 4. For example, the expected change in genotype of X_1 (yield of oil) in Population I when the index based on a weighted average ($b_1 = .8290$, $b_2 = .1073$, $b_3 = -.0507$) is used is:

$$ {}_1\Delta G'_{123} = s\frac{\sum_{i=1}^{3} b'_i g_{i1}}{\sqrt{\sum_{i=j=1}^{3}\sum b'_i b'_j p_{ij}}} = s\frac{\sum_{i=1}^{3} b'_i g_{i1}}{\sqrt{\sum_i b'_i B'_i}} $$

$$ = s\frac{98.98}{\sqrt{177.05}} \qquad = 7.44\ (s)\ \text{pounds,} $$

where

$$ \sum_{i=1}^{3} b'_i g_{i1} = [.8290 \times 128.73] + [.1073 \times 160.607] - [.0507 \times 492.532]. $$

Similarly, the expected genetic changes for the respective characters if other possibilities were considered were determined and are presented in table 8. As expected, the index based on a weighted average gave the more consistent results; however, it

TABLE 8

Expected genetic changes for oil (X_1), protein (X_2) and yield (X_3) when different sources for a selection index are considered

Population	Source of index	Character					
		Oil (X_1)		Protein (X_2)		Yield (X_3)	
		$\Delta G/s$	r	$\Delta G/s$	r	$\Delta G/s$	r
I	Independent culling	7.59	.862	8.33	.859	27.26	.855
	Specific index[1]	8.81	1.000	9.70	1.000	31.88	1.000
	Population I[2]	8.66	.983	9.33	.962	31.09	.975
	Population II[2]	6.78	.770	9.01	.929	26.36	.827
	Weighted average[2]	7.44	.847	9.36	.965	28.38	.890
	Economic worth[2]	6.51	.739	9.06	.934	26.51	.832
II	Independent culling	7.12	.920	13.80	.963	28.80	.997
	Specific index[1]	7.74	1.000	14.33	1.000	28.88	1.000
	Population I[2]	7.29	.942	−.08	−.006	16.35	.566
	Population II[2]	6.00	.775	11.79	.823	27.61	.956
	Weighted average[2]	6.94	.897	9.63	.672	27.24	.943
	Economic worth[2]	5.61	.725	12.28	.857	28.28	.979

[1] Based on yield of character.

[2] Based on relative economic weights for the three characters.

can be further noted that if a selection index were available from only one source of data and applied to a different set of data, the changes for the individual characters appear to be less consistent than if a selection index based on economic worth were considered.

DISCUSSION AND SUMMARY

A criterion for the determination of an average selection index from two or more segregating populations has been presented. The criterion is that the phenotypic weights be so selected that the average genetic advance is a maximum. Since a direct solution of the b_j's was impracticable, the phenotypic variances and covariances and the genotypic variances and covariances were merely pooled to obtain an estimate of the average b_j's. Weights for pooling the respective matrixes were then estimated and a set of phenotypic weights were obtained from the pooled matrixes. One iteration should be sufficient since changes in b_j do not affect appreciably the relative values of W_k and V_{pk}.

Procedures for the calculation of genetic progress expected in genetic worth or in individual characters if selection is to be based on any set of phenotypic weights were outlined. Such weights are considered as constants. A point of interest was that the ratio of the expected genetic advance for a general index to the maximum genetic advance expected for the data utilizing a selection index is the expected correlation between the indexes of phenotypic values obtained with the two indexes.

As noted by JOHNSON (1955, 1957), the two populations are distinct with respect to lodging characteristics and to the genetic correlations involving lodging. In this respect the data are of special interest since one has available data obtained from

distinct genetic populations. Although lodging is not involved in the index, the genetic correlations between X_1, X_2 and X_3 could be affected. If the genetic correlations between the characters of the index depend upon the population sampled, there could be no justification for considering a general selection index for a breeding program in soybeans. From the information presented in tables 5 and 6, it appears that selection indexes may deviate yet give similar information in a selection program. COMSTOCK (1955) has noted the possibility of basing a selection index entirely upon economic worth. Of the factors involved in the construction of a selection index, economic worth is the consistent element. For these two sets of data, the average progress expected, if an index based on economic worth were used, is less than that expected for the optimum index; however, the average genetic advances of these two indexes do not differ appreciably. When the expected genetic change for each character involved in the index was considered, the results were surprisingly similar (table 8), the exception being the case when the index derived from Population I was to serve as the criterion for selection in Population II. Again the index based on relative economic worth appeared quite satisfactory.

In interpreting these results, one certainly must recognize that the environment for the two populations are correlated and that X_1 (yield of oil) contributes principally to the index. Also, additional sources of genetic data are required to evaluate the effects of genetic populations sampled upon expected genetic correlations and the adequacy of the genotype by environment interaction estimate. Additional theoretical information on the sampling errors expected is essential before an adequate evaluation of an average selection index can be made. However, these preliminary results add credence to the assumption that a general selection index in a soybean breeding program is feasible for the criterion of economic worth selected in this paper.

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ANALYSIS OF VEGETATIVE SEGREGATION AND RECOMBINATION IN PENICILLIUM CHRYSOGENUM

G. SERMONTI

Istituto Superiore di Sanità, International Research Centre for Chemical Microbiology, Rome, Italy

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ON the basis of an analysis of vegetative segregation in heterozygous diploids of *Aspergillus nidulans*, PONTECORVO *et al.* (PONTECORVO 1953; PONTECORVO, TARR GLOOR and FORBES 1954) concluded that the segregant nuclei arise as a consequence of two independent processes. The first is mitotic crossing over (STERN 1936), giving rise to diploid nuclei which are homozygous for some of the markers. The other is irregular distribution of whole chromosomes at mitosis; this produces haploid nuclei in a proportion of cases. ROPER and PRITCHARD's (1955) recovery of complementary products of mitotic crossing over provided conclusive evidence for the first of these processes. PONTECORVO and KÄFER (1956) have shown with *Aspergillus nidulans* the practicability of using mitotic crossing over and haploidization for mapping chromosomes.

The two types of vegetative segregant, haploid and diploid, have also recently been identified in *Penicillium chrysogenum* (SERMONTI 1956), so that it is a reasonable assumption that vegetative segregation takes place by means of the same processes in this species. In the same paper the author shows that the spore size of a segregant is diagnostic for its ploidy: diploid spores are about twice the volume of haploid spores.

Observations of spore diameter have not been carried out in the present experiments, and the distinction of strains into haploid and diploid has been based on the phenotype of the segregants and on second-order segregation data.

MATERIALS AND METHODS

Heterozygous diploids. An analysis was made of the three following heterozygous diploids of *Penicillium chrysogenum*:

IV: *63 cy p y / 51 thi pr*

VII: *63 cy p y / 46 me w*

XII: *86 y thi pr / 46 me w*

The Roman numeral is the code number of the diploid. The symbol is made up of the symbols of the two constituent haploids separated by an oblique. Each strain is symbolized by an Arabic number (the strain number) followed by the symbols of the mutations in inverse order of induction. All the strains trace back to the strain Wis. 47.1564; the method of their production and the meanings of the mutation symbols have been described elsewhere (SERMONTI 1954a).[1] The two *y* markers of strains *63*

[1] *cy*, cysteine or methionine requirement; *me*, methionine requirement; *pr*, proline requirement; *thi*, thiamine requirement; *y*, yellow conidia; *w*, white conidia; *p*, absence of penicillin production.

and *86* are regarded as alleles, because diploid *86 y thi pr / 39 arg y* has yellow conidia, and strains *39* and *63* are both descended from the same mutant, *15 y*. Strain *86* is derived from strain *51*.

The heterozygous diploids IV and VII were obtained by selection on minimal media from conidia of the corresponding heterokaryons, and diploid XXI as a sector from a heterokaryon colony. All three diploids have green spores and grow on minimal medium, like the wild type strain Wis. 47.1564. The heterozygous diploidy of the three strains is asserted on the evidence of the present work.

All three diploids were purified by isolation of a single conidium with a micromanipulator (SERMONTI 1954a), and kept on agar slants of complete medium. The cultures were renewed by washing the whole sporing surface and using a sample for transfer. These precautions were devised to reduce to a minimum any bias due to sampling errors in the nuclear population under examination.

Culture media. The following media were employed: a minimal medium (MM), Czapek-Dox as modified by CLUTTERBUCK, LOVEL and RAISTRICK (1932); a complete medium (CM) with corn steep base as described in an earlier paper (SERMONTI 1954a). A modified form of the latter was also used in the second part of the experiment. Its composition was as follows: KH_2PO_4, 1 gm; $MgSO_4$, 0.5 gm; KCl, 0.5 gm; $FeSO_4$, 0.01 gm; sucrose, 30 gm; corn steep liquor, 10 gm; nucleic acid hydrolysate (PONTECORVO 1953), 3 ml; sodium phenylacetate, 1 gm; dl-methionine, 0.05 gm.

Segregant isolation. 1) Colour segregants: Conidia of each diploid are plated on CM; the colonies which grow are observed for colour; all white or yellow colonies are transferred to fresh CM agar for further testing. 2) Nutritional deficiency segregants: Conidia of each diploid are plated on limiting medium (SERMONTI 1954a); colonies which do develop but show only poor growth are collected and subjected to further testing. 3) Segregants for absence of capacity to produce penicillin were selected after total isolation of the colonies obtained by plating conidia of diploid VII on CM. The culture and the titration techniques are described in an earlier paper (CAGLIOTI and SERMONTI 1956).

Characterization of segregants. Colour segregants were checked for uniformity of colour (and purified in cases of nonuniformity) and then classified for nutritional requirements (SERMONTI 1954b). Deficiency segregants were first classified for their specific deficiencies and then transferred to CM agar and examined for their colour, which was uniform in every case.

Strains whose response was open to doubt were transferred to CM agar slants and subjected to more accurate tests. Strains which were to be investigated further were also kept in this way. In all cases in which some doubt arose as to the nutritional requirements of a strain it was subjected to an auxanographic test (PONTECORVO 1949), and the same test was applied to all the strains of critical importance in the present work.

RESULTS

1. *Selection of first-order segregants.* Table 1 shows the rate at which colour segregants were obtained from the three diploids studied and from diploid XIII (CA-

TABLE 1

Rate of occurrence of segregants selected for colour from some heterozygous diploids

Heterozygous diploid	Colonies observed no.	Yellow segregants		White segregants		Ratio of yellow to white segregants
		No.	Percent	No.	Percent	
IV	30,009	76	0.25	—	—	—
VII	14,875	80	0.54	179	1.20	0.45
XIII*	15,130	16	0.11	24	0.16	0.67
XXI*a*	1,648	146	8.86	201	12.20	0.73
XXI*b*	3,788	1	0.026	2	0.05	0.5
Totals†	35,441	243		406		
Averages†			2.38‡ ±2.16		3.40 ±2.94	0.59 ±0.067

* CAGLIOTI and SERMONTI (1956).

† Exclusive of IV.

‡ The standard error is shown as well as the average.

GLIOTI and SERMONTI 1956). Under diploid XXI the segregation rates observed in two different clones isolated from the same sector of a balanced heterokaryon are reported. The first of these, XXI*a*, was pale green; the second, XXI*b*, a more brilliant green. The very wide variability in the rate of occurrence of the colour segregants appears from table 1. The difference in segregation rate between the two clones (*a*) and (*b*) of diploid XXI is particularly striking (21.06 percent in the first and 0.076 percent in the second), although all the markers of the diploid segregated from each clone (see table 2) and the two clones were of about the same age. As is shown by the last column of table 1, the ratio of yellow to white segregants is comparatively much less variable.

Table 2 shows the types of segregant selected from the three diploids IV, VII and XXI (*a* and *b*), with the frequencies of each, subdivided according to the selection method by which they were detected. The figures given for deficiency segregants from diploid VII include five segregants selected for absence of capacity to produce penicillin.

All the markers carried by the parents of each diploid reappear in its segregants. The appearance of the expression of a marker (or mutant gene) in a segregant will be referred to hereinafter as "segregation" of that particular marker (or mutant gene). Caution is required in the evaluation of segregation data, as is suggested by a comparison between the colour segregants and the deficiency segregants from diploid XXI*a*; it will be observed that the rates of types (such as *w me thi* and *y thi pr*) which are detectable either as colour segregants or as deficiency segregants stand in widely different relations in the second and third columns of table 2. This indicates that at least one of the two selection methods is not very efficient. Since the deficiency method requires an elective enrichment the results obtained by this procedure are more likely to be biased.

This last observation, and the presumable occurrence of differences in fitness

TABLE 2

Segregants from heterozygous diploids

Phenotypes	Colour selected	Deficiency selected
(A) Diploid IV:		
63 y p cy/51 thi pr		
(Green prototrophic)		1,335
y p cy thi	1	0
y p cy	75	8
thi pr	n.d.*	8
thi	(1)	2
(B) Diploid VII:		
63 y p cy/46 me w		
(Green prototrophic)		125
y p cy	49	5
y p	31	n.d.
p	n.d.	(5)†
w me	159	20
w	20	n.d.
(C) Diploid XXI:		
86 y thi pr/46 me w		
(clone *a*)		
(Green prototrophic)		289
y thi pr	9	7
y thi	1	0
y pr	129	0
y	7	n.d.
w me thi	131	1
w me	67	6
w	3	n.d.
thi	n.d.	8
(clone *b*)		
w me thi	2	
y pr	1	

The phenotype is indicated by the symbol of the expressed markers.
Deficiency segregants were selected on limiting media.
* n.d. = not detectable.
† Selected for nonpenicillin production out of 476 colonies tested.

between different segregants, make it preferable to neglect (in principle) the figures concerning the frequencies of the segregants in working up the segregation data, and consider only the actual types obtained.

2. *Isolation of second-order segregants.* Genetic analysis of some of the segregants clones was carried out by study of further segregants from them. The selection of second-order segregants was carried out by the same methods as were adopted for first-order segregants. Table 3 shows the phenotypes of some first-order segregants and data of their second-order segregation. The second-order segregants are classified according to the selection method by means of which they were detected.

3. *Ploidy of segregants: inferences based on phenotype.* Ploidy determination of some of the segregants can be carried out on the basis of their phenotypes, on the

TABLE 3

Second-order segregants from heterozygous diploids

First-order segregants analysed		Second-order segregants				
			Colour selected		Deficiency selected	
Phenotype	No.	Phenotype	No.	Total observed	No.	Total tested
(A) Diploid IV:						
63 y p cy/51 thi pr						
thi	1	*thi cy y p*	5	908	9	213
thi	2	*thi pr*	—	2,359	12	66
thi pr	5	—	—	3,448	—	90
cy y p	2	—	—		—	38
thi cy y p	2*	—			—	32
(B) Diploid VII:						
63 y p cy/46 me w						
y p	1	*y p me*	(1)	1,068	1	50
y p	2	*y p cy*	—	1,030	3	30
		y p me	—		3	
cy y p	2	—			—	107
p	2	*p y cy*	6			
		p w me	2	1,786		
		p w	2			
w	2	*w me*			58	93
(C) Diploid XXI:						
46 me w/86 y thi pr						
thi	3	*thi w me*	6			
		thi w	4	2,025		
		thi y pr	45			
		thi y	5			
y thi pr	4	—	—	1,385	—	108
w me	2	—			—	64
y pr†	1	—			—	58

The allele symbols denote phenotypes. Data concerning segregants giving qualitatively similar second-order segregations are pooled together.

Deficiency segregants were selected on limiting media supplemented by the substances required by the first-order segregants in optimal concentrations.

* One was a second-order segregant.

† From diploid XXI*b*.

model of vegetative segregation in *Aspergillus nidulans* (PONTECORVO, TARR GLOOR and FORBES 1954; PONTECORVO and KÄFER 1956). Any statement made in this paper henceforth on possible patterns of gene behaviour in vegetative segregation from diploids of *Penicillium chrysogenum* should be understood in terms of this model.

Following the random segregation of whole chromosomes which leads to haploidization, haploid nuclei with recombinant chromosomes may be formed. This can give rise to phenotypes with recombinant characters only in the case in which genes in repulsion are carried on different chromosomes. Mitotic crossing over (see below) cannot possibly give rise to phenotypically recombinant clones among the first-order

segregants. A phenotype carrying recombinant recessive characters therefore supplies the following information: (*a*) it is haploid; (*b*) the recombinant genes are on different chromosomes; (*c*) if, of two genes in coupling, only one has segregated, the other must be on a different chromosome.

Since mitotic crossing over occurs at the four-strand stage and is followed by equational segregation of the chromatids, a crossing over chromatid segregates either together with the chromatid with which it has exchanged a part, or together with the non crossing over chromatid, the partner of the latter. In the first case, the resultant nucleus remains heterozygous for all genes which were originally heterozygous; in the second case it will become homozygous for only those genes which are on the exchanged portion. This process may lead to partial or total segregation of the genes which are in coupling on the same chromosome arm. Since the rate of mitotic crossing over is so low, the possibility of the occurrence of more than one mitotic crossing over in the same nucleus may be practically ruled out. Once the study of haploid segregants has made it possible to establish linkage groups, the recovery of a phenotype in which segregation takes place of only a part of the genes in coupling belonging to the same linkage group supplies the following information: (*d*) it is diploid; (*e*) the nonsegregant genes are not distal to the segregant genes.

4. *Haploid segregants.* Only two types of recombinants between recessive alleles have been identified in the first-order segregants (table 2), *thi cy y p* from diploid IV and *thi me w* from diploid XXI. There is only one example of the first type, but the frequency of the second is high. On the basis of point (*a*) of section 3, these segregants must be haploid, and the gene *thi*/*THI* must be on a chromosome (I) different from the chromosome or chromosomes carrying the genes *cy*/*CY*, *p*/*P*, *y*/*Y*, *me*/*ME* and *w*/*W*. It must also be on a different chromosome from the one carrying *pr*/*PR*, on account of point (*c*) of section 3. If *pr*, which is in coupling with *thi*, were on the same chromosome as this, it ought always to be present in the above mentioned haploid recombinants. Since no other recombinant phenotype has been found in the first-order segregants of the three diploids examined, all the genes except *thi*/*THI* must probably lie on the same chromosome (II). Other haploid segregants include all those which carry the two markers *thi* and *pr*; since these lie on different chromosomes (see above), they can only segregate together in haploids. These segregants are *thi pr* from diploid IV and *thi pr y* from diploid XXI. The following linkage situation may thus be inferred for the three diploids examined (each line indicates a chromosome; the indicated sequence of the genes on the chromosomes is not significant).

$$\text{IV}\ \frac{thi}{\quad}\ \frac{pr}{(cy\ y\ p)} \qquad \text{VII}\ \frac{\quad}{\quad}\ \frac{(cy\ y\ p)}{(me\ w)}$$

$$\text{XXI}\ \frac{thi}{\quad}\ \frac{(pr\ y)}{(me\ w)}$$

From this diagram it is easy to visualize the possible types of haploid segregants, assuming that haploidization follows a process of irregular distribution of whole

chromosomes. The expected phenotypes have all been found except for *pr* phenotypes from diploid IV (see table 2).

5. *Segregants of uncertain ploidy.* In each heterozygous diploid, chromosome pair II (see diagram above) is always marked on both the component chromosomes, so that each haploid must show all the markers of linkage group II of one or other of the parent strains. Apart from this, the marker *thi* should theoretically be present in half the haploid segregants from diploid IV and XXI (see section 4); in cases in which it is not present there is no phenotypic evidence for the occurrence of haploidization. Strains which belong to this second category, of putative haploids, are the *cy y p* strains segregating from diploids IV and VII, the *me w* strains segregating from diploids VII and XXI and the *pr y* strains segregating from diploid XXI. These strains occur with relatively very high frequencies. Similar segregants could also arise from mitotic crossing over near the centromere of chromosome II if the alleles of each of the above mentioned groups all lie on the same chromosome arm (see above).

6. *Diploid segregants.* These include those in which the thiamine requirement (from diploids IV and XXI) is present alone, for the reasons given at the beginning of section 5, and also include those in which a part only of the markers of linkage group II carried by one parent or the other have segregated (see section 3, point *c*). To this group belong the *y p*, *p* and *w* segregants from diploid VII, and the *y* and *w* segregants from diploid XXI. Diploid segregants probably originate, as already suggested, from a mitotic crossing over process.

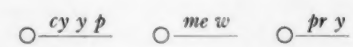
7. *Use of diploid segregants in locating genes relative to the centromere.* A segregating gene following mitotic crossing over is always accompanied by all the genes in coupling with it which lie on the same chromosome arm distal to it relative to the centromere. This assumption makes it an easy matter to fix the sequence of the various markers in coupling relative to the centromere when the phenotypes of the segregant diploids are known.

The three genes *cy*, *y* and *p* appeared in segregant diploids with phenotypes *p* and *y p* and also in the phenotype *cy y p*, whose ploidy is doubtful. Thus the centromere must be located outside the three genes on the *cy* side, they being themselves in the order *cy y p*.

Of the two genes *w* and *me*, only *w* appeared in segregants which are certainly diploid, alongside *me w* phenotypes whose ploidy is doubtful. Thus the centromere must be outside them on the *me* side and the two genes must be on the same chromosome arm.

Of the two genes *y* and *pr*, only *y* appeared in segregants which are certainly diploid, alongside *pr y* phenotypes whose ploidy is doubtful. Thus the centromere must be outside them on the *pr* side, and the two genes must be on the same chromosome arm, i.e. the same one which also carries the genes *cy* and *p*, seeing that both these are also on the same arm as the gene *y*.

The gene groups discussed must therefore have the following locations relative to the centromere:



8. *Information derived from study of second-order segregants.* A strain from which recessive markers segregate is by definition heterozygous for these markers, and thus diploid. All the first-order segregants treated as diploid in section 6 and so far analysed have given second-order segregants (see table 3). In particular, they have been proved to be heterozygous for all the genes between the first-order segregant markers and the centromere of the chromosome to which they belong according to the gene map at the end of section 7, and also to be heterozygous for all genes located on a different chromosome from the one on which the first-order segregant markers lie. This is precisely what might be expected if the segregant diploids are assumed to originate from mitotic crossing over.

Second-order segregants may supply information on the relative position of markers in repulsion lying on the same chromosomes. The *p* segregants from diploid VII give second-order segregants for *w* and *me*, while *y p* segregants give *me* segregants but no *w* segregants. This is compatible with the hypothesis that the gene *w/W* is between the genes *Y/y* and *P/p*, and the gene *me/ME* between *Y/y* and the centromere:

$$\bigcirc\ \frac{me\ \ Y\ w\ \ P}{}$$
$$\bigcirc\ \frac{}{ME\ y\ W\ \ p}$$

A homozygous *p/p* segregant might remain heterozygous for *w/W*, but a homozygous *y p/y p* segregant must be also *W/W* homozygous, though it might still be *me/ME* heterozygous. These points are made on the assumption that, as in *Aspergillus nidulans* (PONTECORVO 1953), *w* is epistatic to *y* and not *vice versa*.

9. *Genotypes of some segregant diploids.* The genotypes of some of the segregant diploids can be completely deduced from the second-order segregation data (see also section 8). This applies to the *p* and *y p* segregants from diploid VII, whose genotypes must be as follows:

$$\bigcirc\ \frac{(CY\ me)\ Y\ w}{} \times \frac{\ \ p}{}$$
$$\bigcirc\ \frac{}{(cy\ ME)\ y\ W} \times \frac{}{\ \ p}$$

$$\bigcirc\ \frac{(CY\ me)}{} \times \frac{y\ W\ p}{}$$
$$\bigcirc\ \frac{}{(cy\ ME)} \times \frac{}{y\ W\ p}$$

and also to the *thi* segregants from diploid XXI, whose genotype must be:

$$\bigcirc\ \text{——} \times \frac{thi}{}$$
$$\bigcirc\ \text{——} \times \frac{}{thi}$$

$$\bigcirc\ \frac{(PR\ me)\ Y\ w}{}$$
$$\bigcirc\ \frac{}{(pr\ ME)\ y\ W}$$

The segregants mentioned must be derived from mitotic crossing over processes in correspondence with the points marked in the three diagrams. The markers shown as in the homozygous state are all phenotypically expressed in the first-order segregants, the markers shown as in the heterozygous state all emerge in the second-order segregants and the markers whose wild type allele is present in the homozygous state do not appear in the phenotype of either first or second-order segregants.

10. *Proposed map for seven genes.* No information has been gained on the position

relative to one another of the genes *pr*, *cy* and *me*, which however must all be between gene *y* and the centromere of chromosome II—the first two on the basis of the first-order segregation results (section 7), and the third on the basis of the argument in section 8. The order of the genes *y*, *w* and *p* has also been fixed in section 8. They are distal to the genes already mentioned on the same arm of chromosome II, in the order given. Gene *thi* is on a different chromosome (section 4). These findings may be summed up in the following diagram:

$$\circ\!\!-\!\!\overline{\quad thi \quad} \qquad \circ\!\!-\!\!\overline{\quad (cy,\ me,\ pr)\ y\ w\ p \quad}$$

11. *Coupling and repulsion.* The markers *thi* and *pr* are in coupling with *y* in diploid XXI, and in repulsion with it in diploid IV. Since *thi* is on a different chromosome from *y*, and *pr* is on the same arm as *y*, the situation particularly favours a check of the assumptions which have been made. The situation may be illustrated diagrammatically as follows:

$$\text{IV}\quad \frac{\circ\ thi}{\circ\ +} \qquad \frac{\circ\ pr\ +}{\circ\ +\ y} \qquad\qquad \text{XXI}\quad \frac{\circ\ thi}{\circ\ +} \qquad \frac{\circ\ pr\ y}{\circ\ +\ +}$$

The data concerning segregation from the two diploids are precisely what would be expected (table 2): *y* segregates sometimes with and sometimes without *thi* in the two diploids IV and XXI (and the same is true of *thi* in its relation to *y*); on the other hand, *y* never segregates with *pr* in diploid IV, and in diploid XXI it generally segregates with *pr* but sometimes without it (mitotic crossing over between *pr* and *y*).

DISCUSSION

This paper is based on the model of vegetative segregation in *Aspergillus nidulans*, as formulated by PONTECORVO, TARR GLOOR and FORBES (1954). Since the data might not in fact have suited the model, they supply some information on the applicability of this model to the species. Two types of evidence are particularly unequivocal; in the first place, all the strains which were analysed on the assumption (based on the phenotype) that they were diploids turn out to be diploids on the evidence of their second-order segregations, and in the second place, expectations concerning the behaviour of some genes which are in coupling in one diploid and in repulsion in another are fully satisfied. The experiments carried out have given fairly reliable results on the location of some genes, but on the other hand labour under serious difficulties. The chief of these is the necessity of obtaining an adequate estimate of the frequency of the segregation process. Such an estimate is presumably biased by at least three sources of selective pressure on the material: 1) differences in fitness in the segregant clones; 2) the clonal distribution of the segregant nuclei; and 3) variations in the practical detectability of the segregant strains.

The only way to overcome the first of these difficulties would be the application of methods which allowed immediate isolation of nuclei deriving from segregation processes. Correction of the bias by means of reconstruction experiments intended to estimate the fitness of various clones is laborious and doubtful, since the selection

may act both between nuclei on the same heterokaryotic hypha and between different hyphae.

The second difficulty could be obviated by selecting not more than one segregant of each type from a large number of heterozygous diploid colonies of monoconidic origin (PONTECORVO, TARR GLOOR and FORBES 1954). A precaution such as this would vastly increase the laboriousness of the experiments and would be of comparatively little effect unless the first source of error was reduced. In any case, segregant isolation from small samples taken from large numbers of conidia carefully mixed, as used in the present experiments, makes it unlikely that the same segregant clone would be isolated more than once.

The third difficulty crops up when segregant isolation is obtained after enrichment of the segregants by some kind of artificial selection method. The difficulty could be obviated by total isolation of the population in which the segregants are looked for, but this expedient again would increase the laboriousness of the work to an excessive degree.

Further work on gene location in *Penicillium chrysogenum* by study of vegetative segregation from heterozygous diploids will not make appreciable progress until markers are available allowing for very efficient segregant detection; generally speaking, the type of marker required is one which confers on haploid or homozygote strains a very definite advantage over such strains which are heterozygous for this marker. Markers such as recessive suppressor of adenine requirement and incompletely dominant resistance to acriflavine (PONTECORVO and KÄFER 1956) are of this type.

The gradually increasing body of knowledge of the genetic map of Penicillium will make it continually easier to design new experiments for this purpose, since reference points for the location of new genes will become available and criteria will be set up for the distinction of strains into haploid and diploid on the basis of phenotype.

SUMMARY

An analysis of vegetative segregation has been carried out in three heterozygous diploid strains of *Penicillium chrysogenum*, using as model the vegetative segregation of *Aspergillus nidulans* (PONTECORVO, TARR GLOOR and FORBES 1954). The results do not contradict the model in any case. On the basis of the segregation data of seven markers it has been possible to identify two (mitotic) linkage groups and the sequence of a number of genes.

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INDUCED CHANGES IN FEMALE GERM CELLS OF DROSOPHILA. IV. DEPENDENCE OF INDUCED CROSSOVER-LIKE EXCHANGES IN OOCYTES AND OOGONIA UPON X-RAY INTENSITY[1]

IRWIN H. HERSKOWITZ AND S. ABRAHAMSON[2]

Department of Zoology[3], Indiana University, Bloomington, Indiana

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A number of studies have shown that in Drosophila (see HERSKOWITZ 1953 for references) the rate of exchanges between exactly homologous loci, crossovers, depends upon sex and other genotypic factors, age of female, stage in gametogenesis, temperature, nutrition, and degree of hydration (IVES, FENTON, YOST and LEVINE 1953; LEVINE 1955). For recent data and a review of induced crossing over see WHITTINGHILL (1955). The effect of temperature on crossover rate has been interpreted as being due to a modification of the spontaneous crossover process, and the effect of X-rays on such exchanges has been similarly explained on the basis of four lines of evidence. (1) The X-ray effect is apparently a direct one, in that it seems both to increase linearly with dosage (PARKER 1948) and to be independent of radiation intensity (WHITTINGHILL 1951). (2) The cells and the cell stages affected by X-rays and temperature are similar (for example, when adult females are treated no clear and consistent effect on crossover-like exchange frequency was noted in eggs first laid, but one appeared in later-laid eggs). (3) The pattern of change in the frequency of crossovers along the length of a chromosome is similar for X-rays, age and temperature, the effect being greatest closest to the centromere (MULLER 1925, 1926). (4) There is no greater tendency, following X-ray treatment, for lethals to be associated with points of crossing over than of non crossing over (PATTERSON and SUCHE 1934; FRIESEN 1937). If the induced crossover-like exchanges were due to breakages occurring at random at breakable places along the chromosome they frequently would be expected to occur between nearby but not homologous loci—causing the recombinant strands, properly called "pseudocrossovers" (a term suggested by H. J. MULLER), to have small duplications and deficiencies, the latter often acting as recessive lethal mutations. Because the frequency of recessive lethal mutations declines in successive eggs laid after irradiation (KING 1952) while the effect on crossover-like exchanges is known to increase and reach a peak in eggs laid later, this also has argued in favor of the exchanges in later laid eggs being between homologous loci, e.g. crossovers.

It became desirable to retest the effect of X-ray intensity on crossover and crossover-like exchanges in occytes and oogonia since the earlier work had not employed as great an intensity difference as was used recently in demonstrating that half-translocations were intensity dependent (HERSKOWITZ and ABRAHAMSON 1956a). It was also apparent from the half-translocation work that one might obtain, amongst

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[2] Eigenmann Fellow in Zoology. Present address: Department of Genetics, University of Wisconsin, Madison, Wis.

[3] Contribution No. 637.

eggs first laid, exchanges between homologous chromosomes which would appear to be crossovers but which would be, in reality, pseudocrossovers or half-translocations following multiple chromosome breakages. It may be mentioned in this connection that, in the data given in some of the earlier papers of MAVOR and SVENSON (1924, 1925) and of others since that time (e.g. PARKER in discussion of paper by WHITTINGHILL 1955), crossover-like exchanges seemed to be increased by radiation in eggs laid within the first four days after treatment of the adult female (cells which, when irradiated, were oocytes presumed to be past the stage of spontaneous crossing over). However, the number of adults from such eggs were usually not sufficient, because of the high X-ray-induced egg mortality, to permit the conclusion that there was really an increase and it was assumed by most Drosophila workers (see MORGAN, BRIDGES and STURTEVANT 1925, p. 104–105) that there was no increase among eggs laid during the first five days after any treatment.

The present study was designed therefore to retest the effect of radiation intensity on the rate of crossover and crossover-like exchanges in eggs laid in successive periods with particular reference to the rate obtained among flies derived from the eggs first laid. The experiments will be discussed as to their bearing on the mechanism of action of X-rays in producing crossovers and pseudocrossovers. Some of the present results have been referred to earlier (HERSKOWITZ and ABRAHAMSON 1956b).

MATERIAL AND METHODS

Drosophila melanogaster was used in this study. Virgin females were collected, aged 4–8 days, and were either not irradiated or treated with X-rays while motile and well aerated, at 20 ma at a peak of 200 kv with 1 mm Al filtration. After this they were placed without additional etherization into pint milk bottles containing culture medium and an excess of males. The parents were transferred to new bottles periodically.

In both Experiments I and II the females were $y \cdot Dp\ sc^{v1}\ y^+/y^2\ v\ f\ car$ (the duplication, $Dp\ sc^{v1}\ y^+$, is attached beyond the centromere of an X chromosome having otherwise a normal structure and containing the genes *y* and sc^+ in their usual distal position), and the males $Y^+/y^2\ v\ f\ car$ (except for a small group which was $Y^+/y\ cl^6\ f\ car$). The dosage of about 2940r was given either in a concentrated treatment in two minutes five seconds at 1404r/min, or in a protracted treatment at 23r/min in 16 irradiations each eight minutes long with 20-minute stops between successive irradiations (requiring seven hours seven minutes to deliver). In both experiments the number of females per bottle was ten for the control, 40 for the concentrated and 32 for the protracted series. In Experiment I the duration in days for successive broods was 2, 1, 2, 3, 3, 3, and in Experiment II 2, 1, 1, 1, 3, 3, 3.

The numbers and phenotypes of all F_1 flies were determined after periodically removing them from the bottles. Each bottle was scored through the 18th day from the time parents were introduced, at which time very few or no flies were eclosing.

The temperature following treatment was 25–27°C throughout the experiments.

RESULTS AND DISCUSSION

Table 1 gives the frequencies of exchange between car^+ and y^+ among 45,537 flies scored in Experiment I and 87,560 flies scored in Experiment II in different broods of

TABLE 1

Frequency of exchanges between car$^+$ *and* y$^+$ *among* F_1 *for different treatments*

	Brood 1	2	3a	3b	4	5	6	Total
	Days 1–2	3	4	5	6–8	9–11	12–14	
Experiment I								
Unirradiated (control) (4 bottles/brood)								
No. F_1	1645	1144	1707		2792	2002	2222	11,512
Percent exchanges	3.5	3.2	3.2		2.2	1.8	1.5	
Protracted dose (5 bottles/brood)								
No. F_1	1191	2044	2941		4070	3691	4562	18,499
Percent exchanges	4.4	4.2	3.8		3.2	3.7	5.9	
Concentrated dose (5 bottles/brood)								
No. F_1	988	1162	2343		3269	3535	4229	15,526
Percent exchanges	5.2	5.5	5.3		6.1	7.4	9.2	
Experiment II								
Unirradiated (control) (10 bottles/brood)								
No. F_1	7211	2904	2331	1799	5725	5171	5067	30,208
Percent exchanges	3.1	3.2	3.4	2.4	2.2	1.7	2.1	
Protracted dose (9 bottles/brood*)								
No. F_1	3749	1880	2123	2823	6072	6538	6143	29,328
Percent exchanges	4.6	3.6	3.9	3.5	3.7	4.1	5.9	
Concentrated dose (10 bottles/brood)								
No. F_1	2621	1398	1574	2720	6259	6766	6686	28,024
Percent exchanges	6.3	7.8	5.3	5.2	5.3	7.2	8.1	

* Except in broods 3a and 3b where there were eight.

eggs oviposited following the different treatments. In every brood in both experiments, the control rate of exchange was lowest, the one for the protracted irradiation was intermediate, and that for the concentrated irradiation highest. A decrease in crossover frequency in the control series as the female parents aged, long ago established by BRIDGES (1915) and confirmed by PLOUGH (1917) and others, was found here also.

For those bottles in Experiment I in which $Y^+/y^2\ v\ f\ car$ males were used as parents, exchanges involving the *v-f* and the *f-car* regions were scored as well as those between *car*$^+$ and *y*$^+$. These data are presented in table 2. For the *f-car* region the protracted and concentrated doses gave higher values than the control in broods 2, 4, 5, 6, while for the *v-f* region broods 2, 5, 6 and 2, 4, 5, 6 were higher in the protracted and concentrated treatments, respectively, than in the control. These data are consistent with those of MULLER (1925, 1926) and of later workers in that the greatest effect on "crossovers" is found in the region tested which lies closest to the centromere.

TABLE 2

Exchange percentages for various regions of the X chromosome in those bottles in Experiment I in which mating was with Y^+/y^2 v f car *males**

Region		Brood 1	2	3	4	5	6
		Days 1–2	3	4–5	6–8	9–11	12–14
car—centromere	C	3.6	3.7	3.7	2.1	2.0	1.4
3.5	(Total F_1)	(1314)	(972)	(1439)	(2253)	(1614)	(1836)
	D	4.5	4.1	3.8	3.2	3.6	6.0
	(Total F_1)	(1156)	(1938)	(2737)	(3699)	(3383)	(4250)
	I	5.2	5.3	5.3	6.1	7.2	9.2
	(Total F_1)	(973)	(1115)	(2266)	(3078)	(3379)	(4066)
f—car 5.8	C	6.7	3.4	4.9	3.5	2.6	2.7
	D	5.1	4.5	4.6	4.1	3.6	4.5
	I	3.8	4.8	4.2	4.0	4.3	5.5
v—f 23.7	C	23.4	20.5	22.8	23.1	20.8	20.4
	D	21.3	21.8	21.9	21.5	21.4	24.0
	I	21.5	21.7	21.4	23.6	22.8	23.0

* No. bottles per brood was 2, 4 and 4 for the unirradiated (C), protracted (D), and concentrated (I) treatments, respectively. Italicized numbers are the standard crossover map distances.

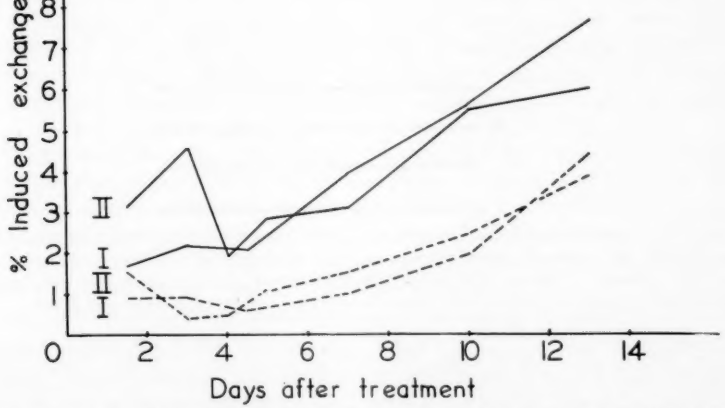

FIGURE 1.—Induced frequencies of exchange between car^+ and y^+ in groups of eggs laid in different periods after concentrated (solid line) and protracted (broken line) treatments in Experiments I and II.

Figure 1 presents the induced frequencies of exchange (obtained by subtracting, from the rate after irradiation, the control rate for the same brood) between car^+ and y^+ in eggs laid in different broods in both experiments, using the data in table 1. Whenever an egg-laying period was longer than one day the middle time is used, for convenience, to represent the time at which all the eggs were oviposited.

That the present experimental results are due to either or both a differential delay in oviposition or differential resorption of oocytes in different treatments is extremely unlikely in view of a discussion by HERSKOWITZ (1957).

GENERAL DISCUSSION

The cells tested in these studies were, at the time of irradiation, oocytes, with chromosomes in tetrad condition, and oogonia. Figure 2 shows schematically the tetrads of the X chromosome and of an autosome as they might be arranged in the oocyte, indicating the relative positions of the yellow and carnation loci. There are

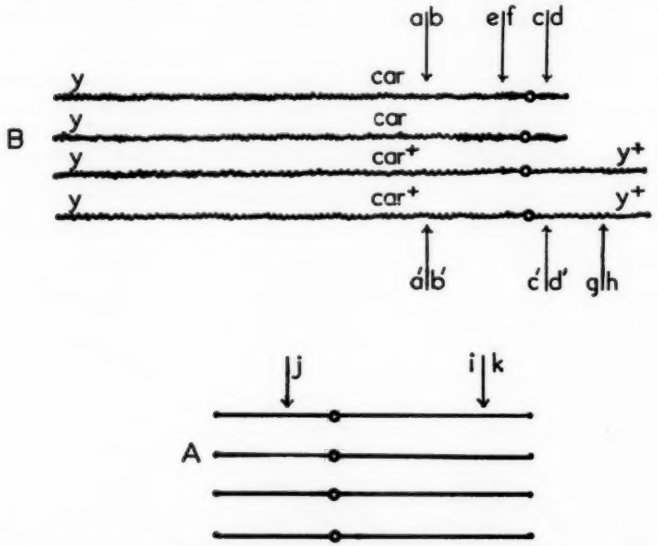

FIGURE 2.—Diagrammatic scheme of tetrads of the X chromosome (wavy lines) and of an autosome (straight lines) showing points of breakage or exchange (arrows) whereby *car*$^+$ and y^+ can change their linkage. Details are given in the General Discussion. ● = telomere, ○ = centromere. A letter and its prime (such as *a* and *a'*) represent identical positions on two different strands.

several ways in which mature eggs bearing either y^+ or *car*$^+$, but not both, could be produced. One of these dominant markers could become a recessive mutant through gene mutation or become lost by deletion from the X chromosome by two or more breakages in the same strand, or y^+ or *car*$^+$ could be gained by reverse mutation or by an insertion, following multiple breakage, in one of the two strands not originally containing these genes. These events however are relatively rare so they will be neglected in the rest of the discussion. (By far the most frequent is deletion of the y^+ which, if like that of y^+ from an X chromosome containing *sc*^{8} in sperm, would have a frequency at 5000r of about 0.5 percent, see VALENCIA and MULLER 1949. However, the rate for *Dp sc*^{v1} y^+ in irradiated oocytes might be lower.) There are

several other ways *car*$^+$ and *y*$^+$ may relatively frequently change their linkage in the present experiments (fig. 2). (1) A crossover may be produced between exactly homologous loci, either between *car*$^+$ and the centromere (producing crossover strands by *a* joining to *b'* and *a'* to *b*), or, less frequently, between the centromere and the right hand telomere (producing *cd'* and *c'd* strands). (2) A pseudocrossover (exchange between nearby but non-homologous loci) could occur which would produce small deficiencies or duplications. If in figure 2 the right side of a breakage in one strand joined to the left side of a breakage in another strand and the two breakages involved both occurred on the same side of the centromere, eucentric rearrangement would result producing *a'f* and *eb'* or more rarely *ch* and *gd*, but if the breakages involved were on different sides of the centromere, one of the two strands produced would be dicentric (for example *c'f*) the other acentric (*d'e*)—the latter certainly and the former probably would not be capable of continued cellular transmission when included in the female pronucleus. Other pseudocrossovers would be those in which, in the diagram, the right side of a break joined to the right side of another break in another strand or a left side to a left side. Such joinings would produce, when the two breakages were on different sides of the centromere, for example, *ca'* (attached-X's) and *db'*, or *eg* (attached-X's) and *hf*, while when the breaks were on the same side of the centromere an acentric (*ea'*) and a dicentric (*fb'*) would be produced which would, like the other aneucentrics already mentioned, probably not be able to survive. (3) These two genes also would no longer be connected in any gamete containing a eucentric half-translocation which removed *y*$^+$ from its linkage to *car*$^+$. For by such a half-translocation one could obtain a gamete either carrying an unbroken *car*-containing chromosome (B) plus a broken autosome which was capped by a fragment containing *y*$^+$ (*ih*), or a gamete carrying a broken *car*$^+$-containing chromosome made eutelomeric by being capped by a broken piece of autosome (*c'k*) plus a normal autosome (A) from that tetrad.

The following discussion attempts roughly to estimate the contribution of crossing over, pseudo crossing over, and half-translocation to the induced exchanges. First will be considered exchanges in the eggs laid within the first four days of treatment, which were usually oocytes as far developed as metaphase I at the time of irradiation, and second exchanges in eggs laid six or more days after irradiation, which were usually early oocytes or oogonia at the time of treatment.

Both pseudo crossing over and half-translocation require one break in each of two different chromosome strands. Since the frequency of half-translocations (and also of recessive lethal mutations) is highest in the eggs laid the first days after irradiation and declines in eggs oviposited later (HERSKOWITZ 1954; HERSKOWITZ and ABRAHAMSON 1956a; ABRAHAMSON 1956) it is likely that pseudocrossover frequency follows the same pattern and that some of both types of mutations occurred in the first laid eggs in the present experiments. An estimate of the frequency of half-translocations induced by irradiation of the late oocytes in the present experiments can be obtained indirectly from data of another experiment. When females homozygous for *y sc*^{5}·*Dp sc*^{v1} *y*$^+$ were irradiated with 4250r delivered intensely about 0.6 percent *y*$^+$-deficient half-translocations and/or deficiencies of *sc*^{v1} *y*$^+$ were obtained among F_1 from eggs laid within four days of the irradiation (MULLER and HERSKOWITZ

TABLE 3

Incidence of half-translocations among y^+-containing exchanges (No. half-translocations/no. exchanges tested)

Treatment	Eggs laid on day(s)	
	1–4	5 and later
Unirradiated	0/22	0/40
Irradiated intensely	2/14	0/68

1956). Since the dose used in the present work was about 3000r, and half-translocation frequency is known to decline exponentially about as the $\frac{3}{2}$ power of the dose as the dose decreases (HERSKOWITZ 1954; ABRAHAMSON 1956), one would expect not much more than half (.58) the frequency obtained with 4250r to be produced per y^+-bearing strand in the present experiments. Because, in the present experiments, only two of the four strands in the X chromosome tetrad carry y^+, the frequency might be reduced again by one half, making the expected frequency of such half-translocations approximately 0.18 percent. It would seem on this basis that in the present experiments, where the induced rate of exchange is about 2.5 percent, only a relatively small part would be expected to come from half-translocation. This reckoning assumes that when a tetrad contains only two such y^+-bearing strands, having no homologous region in the other two strands readily available to pair with, there is no greatly increased chance for half-translocation per y^+-bearing strand as compared with that in a tetrad whose strands all bear the y^+-containing right arm.

To obtain a more direct estimate of the contribution of half-translocation to the total induced frequency of seeming crossovers of early-laid eggs, the presence of half-translocations was tested for in the following way. Some y^+-containing exchange males were crossed individually to virgin $y\,f$: = females. If the y^+-containing piece was a half-translocation the cross should produce some gray-bodied forked-bristled daughters. Such a cross of exceptions from eggs laid the first four days showed 2 of 14 tested individuals from an intense irradiation and none of 22 tested from the controls to be half-translocations (table 3). This means, assuming car^+-containing exchanges are half-translocations as frequently as are y^+-containing ones, that about one seventh of the overall exchange rate in the intensely irradiated series for eggs laid early (about six percent) were half-translocations, or about 0.9 percent. Since the induced rate of exchange in late oocytes is about 2.5 percent, this more direct method of estimation indicates about 36 percent of this 2.5 percent is likely to be due to half-translocat ons the rest due to pseudo crossing over, crossing over and a few deletions of y^+. It may be noted that one case of pseudo crossing over which had arisen in an egg deposited within four days of irradiation has already been genetically proven by MULLER (1954), and others are to be reported (MULLER and HERSKOWITZ, in manuscript).

Because the number of y^+ exchanges tested was limited it would be premature to conclude that the y^+-containing strand is more likely to half-translocate when heterozygous than when homozygous. It should be noted that the testing of y^+-containing exchanges would be biased against detecting half-translocations were these considerably less viable than the reciprocal class of exchange. That this is not the case is clear from the fact that in the first four days of egg-laying approximately

the same ratio of *car*$^+$ to *y*$^+$ exchanges was obtained amongst females in the intensely irradiated series (118/121, or 0.98:1) as was obtained in the controls (125/124, or 1:1). These data are consistent, therefore, with the conclusion that in late oocytes, as represented by eggs laid the first four days following irradiation, the greater part of the induced crossover-like exchanges are pseudocrossovers and/or crossovers.

The relatively high frequency of pseudocrossovers plus crossovers, as compared with gross structural changes involving the same region, attests to the importance of the initial proximity of the "broken" strands in determining which ones shall undergo recombination rather than restitution of "broken" ends.

The data prove the induced exchanges between *car*$^+$ and *y*$^+$ are intensity dependent, as would be expected both for half-translocations and pseudocrossovers since they result from independently produced multiple chromosomal breakages.

No clear effect was observed on pseudocrossover plus crossover frequency in the *v-f* or *f-car* regions tested. This is not surprising, so far as *v-f* is concerned, once it is considered that the induced exchanges occurred in strands which previously had an opportunity to undergo a high frequency of spontaneous crossing over. Moreover, the smaller the crossover region and the greater its relative "breakability" when X-rays are applied, the larger the induced exchange rate would be. Thus, "breakage" followed by structural change is relatively very frequent between *car* and the centromere (only 3.5 units apart), much less frequent in the *f-car* region (5.8 units long), and while frequent in the *v-f* region taken as a whole, it is masked by being added to the normal crossing over rate of 23.7 percent. It is not entirely added, however, for in this region 23.7 percent of the induced exchanges would be undetected because they made what appear to be nonrecombinant strands out of strands which had previously undergone spontaneous crossing over.

Consider next the contribution of half-translocations, pseudo crossing over, and crossing over to the induced "exchange" rate in early oocytes and oogonia. The contribution of half-translocations is probably negligible because the rate of this event is known to decline in eggs laid after the first few days. Moreover, none of 68 exchanges of the *y*$^+$-containing type from eggs laid five or more days after the intense treatment could be demonstrated to be half-translocations (table 3) when tested in the manner already described. Pseudocrossovers also probably contribute little since, as already mentioned, the recessive lethal rate and the structural change rate in general (see MULLER, VALENCIA and VALENCIA 1949) decrease sharply in later laid eggs. It is concluded then, that the induced exchanges in early oocytes and in oogonia are almost entirely crossovers.

The data from later broods confirm the fact that X-ray treatments given both intensely and dilutely produce a definite increase in crossing over, which is, as is well known, greater the closer the region is to the centromere. The fact that crossing over is increased more by an intense than a protracted dose proves, further, that X-ray-induced crossing over is a multihit event. Since there is an intensity effect the more immediate X-ray effect is a temporary one. This gives evidence that the induced crossing over probably takes place in effect (i.e. is determined) very soon after the irradiation is administered. However, since the X-ray effect persists in eggs laid over a period of a week and more, the "decision" or the inclination to crossover can be brought about in cell stages earlier than the one to which spontaneous meiotic crossing

over is usually limited. Moreover, the effect is precise in producing exchange between two strands at what is apparently exactly the same place in both although the "hits" could not have been localized in this way. Of course, the points of exchange in X-ray-induced crossing over could be inexact insofar as any nongenetic material is concerned—but the evidence is that it must be exact from the standpoint of producing no deficiencies of genes or gene parts whose absence would have lethal or visible effects. The X-ray effect is very likely, therefore, not directly on the genes which undergo the exchange, but is more indirect. Experiments to test the effect of oogonial dehydration on X-ray-induced crossing over have been performed (HERSKOWITZ 1956) and showed a definite enhancing effect, also suggesting that X-rays act indirectly on the chromosome in increasing crossing over.

SUMMARY

Females heterozygous for X chromosomes, one of which was normal in length and carried y^2 *v f car* while the other carried *y* and car^+ in the long left arm and y^+ attached to the right arm as part of $Dp\ sc^{v1}$, were irradiated intensely or protractedly or not irradiated, crossed to males bearing the recessive alleles of the marker genes, and the frequency of exchange between the markers determined in F_1 from successive groups of eggs laid. The greatest increase in exchange frequency was for the car^+-y^+ region containing the centromere.

The frequencies of exchange in this region were, in all broods of eggs, highest after the intense irradiation, lower after the protracted one, and lowest for the control. For example, in eggs laid 1–2 days after treatment the rates were, respectively, 5.2 percent, 4.5 percent and 3.6 percent, while for days 12–14 the rates were, respectively, 9.2 percent, 6.0 percent and 1.4 percent. This proves that the events responsible for intensity-dependent exchange depend upon more than one hit and that the process whereby the effects of these hits cooperate to induce the occurrence of the exchange is localized in time.

Amongst eggs laid the first four days after treatment the induced exchanges are attributed partly to half-translocations, but mostly to crossovers or pseudocrossovers (exchanges between different strands of a tetrad involving breakages at loci which are nearby but not necessarily homologous). In eggs laid later the induced exchanges are probably almost entirely crossovers (exchanges between exactly homologous loci). It is inferred that the effect of X-rays in increasing crossing over is not directly on the chromosome loci at which exchange occurs.

The relatively high frequency of pseudocrossovers plus crossovers, as compared with gross structural changes involving the same region in late oocytes, attests to the importance of proximity of the strands undergoing exchange in determining which ones shall undergo recombination rather than restitution.

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THE POSSIBILITY OF FORECASTING THE RELATIVE RATE OF INDUCED LOSS FOR ENDOSPERM MARKERS IN MAIZE[1]

A. C. FABERGÉ

Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee

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Importance of selection in mutation studies

MANY studies of induced mutation have consisted essentially in irradiating individuals or gametes, carrying dominants, and crossing them to individuals with the corresponding recessives. One thus obtains mutation rates for single loci, the classical investigations with Drosophila and mice being examples. Maize lends itself to the same technique, either seedling or endosperm characters being used, and today the data available in maize are as extensive as in any higher organism. While experimental procedures are superficially alike, there are great differences in the amounts of selection in different organisms. Thus in Drosophila, deficiencies greater than about 50 salivary gland bands are eliminated as dominant lethals, together with all chromosome structures unable to go through mitosis normally. The gametophyte in maize also tolerates only rather small deficiencies, and maize mutants taken through a zygotic generation are, equally, highly selected.

Maize endosperm on the contrary probably presents almost no selection barrier, and all or nearly all the products of the mutagenic process can be examined. In the usual experiments, the triploid endosperm carries two normal chromosomes from the female parent, and these cover any viability defect in the treated chromosome. These various degrees of associated selection have drastic consequences on the classes of mutants recovered, and probably are a principal cause of differences observed when various organisms are compared in mutation experiments.

Chromosomal nature of marker losses in maize endosperm

That losses of dominant markers in maize endosperm are chiefly chromosomal, rather than genic, has been accepted by most workers for a long time. It is quite directly and simply demonstrated by analyzing combinations of markers, in an experiment where several are present simultaneously in one arm (FABERGÉ 1956a). Essentially, it is then observed that multiple losses appear at a frequency of the same order as single marker losses. When multiple losses are examined in detail, it is found that they invariably consist of adjacent or *consecutive* markers. Nonconsecutive multiple losses are not observed. These would be expected to appear at a frequency equal to the product of the frequencies for individual markers, and so are too rare to be observed in an experiment of the usual size. MCCLINTOCK's (1938a, 1941) discovery of, and investigations on the breakage-fusion-bridge cycle have made it possible to interpret endosperm mosaics in terms of the underlying chromosome mechanics.

In FABERGÉ (1956a) it is shown that the relative frequencies of various kinds of losses and mosaics can be explained with reasonable consistency by some simple

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assumptions about chromosome behavior. The effects consist essentially of *losses* covering large areas or whole kernels, and finely divided up mosaics. It is proposed to use the word *loss* to designate the phenotypic effect without any implications about the underlying mechanisms. In symbols, we shall write L(A) for the loss so defined of the marker A. Many losses may be due to true segmental deficiencies; in such cases, we shall write Df(A). Finely broken up mosaics are caused chiefly by breakage-fusion-bridge cycles generated from a free, broken chromosome end. A critical demonstration of a B.-F.-B. cycle requires at least two markers on one arm. Because the proximal marker is never lost earlier than the distal in cell lineage, the resulting mosaic is characteristic. It constitutes a curiously literal though trivial instance of the Set Theory relation of partition, so that one can always write: proximal loss $\subseteq$ distal loss. In fact, illustrations closely resembling endosperm mosaics can be found in elementary expositions of Sets. The free end generating a B.-F.-B. cycle may result directly from the mutagen, or may follow breakage of a dicentric or ring chromosome. In the case of simple, directly produced breaks, the distal segment is lost and, if carrying a marker, results in an observed loss, while the proximal part generates a B.-F.-B. cycle. Because the B.-F.-B. cycle gives the order of the markers, some types of incomplete rearrangement can be identified. We shall write Cy (AB) for a B.-F.-B. cycle initiated in the segment AB.

Influence of more complex aberrations

The analysis of more complex aberrations given in abbreviated form in FABERGÉ (1956a) shows that they are numerically not very important, and their contributions to simple loss rate even less so. It appears that in maize endosperm, an overwhelming majority of induced exchanges occur within an arm. For these reasons, it is legitimate to analyze losses as though the only elements are simple segmental deficiencies, and terminal losses of the kind generating B.-F.-B. cycles. This is recognized to be an approximation, but the error made is probably no greater than the uncertainties of cytological location of markers.

Proportionality of breakage to pachytene length

It can be directly shown (FABERGÉ 1956a) that the points of breakage initiating B.-F.-B. cycles in 9S are produced at a rate closely proportional to pachytene length. The relative frequencies of various deficiencies can be explained by assuming that the breaks that generate them are also produced in proportion to length.

LONGLEY (1950) plotted the positions of 1176 induced breaks in translocations, throughout the complement. These also occur at a frequency roughly proportional to pachytene length. LONGLEY devotes attention to departures from exact proportionality in his data and demonstrates an excess of breaks in regions near centromeres. However, as a whole, these departures are not large. KONZAK and SINGLETON (1956b) report an excess of breaks in the region *Wx*-SFA. In their analysis, however, no allowance is made for the fact that B.-F.-B. cycles were excluded if they occurred in the region distal to *Wx*, but those occurring in *Wx*-SFA contributed to the simple loss class. Their marker system would make it difficult to apply the algebraic relationship used in FABERGÉ (1956a) to allow for this. The very detailed data available for Drosophila salivary gland chromosomes, gives an essentially similar picture: if the

euchromatic part of the X chromosome be divided in small regions, large departures from proportionality appear, whereas if longer regions are taken, these irregularities are smoothed out (KAUFFMAN 1946). In this connection, the question might be raised whether it is preferable to speak of an excess of breaks in some segment, rather than of greater condensation of that segment at pachytene. It is at least conceivable that rate of breakage may be a better measure of physical length in the resting stage, when radiations are applied.

Thus, approximate proportionality of breakage to length has been demonstrated in maize on two scales: that of the entire complement, and that of a single short chromosome arm.

Possibility of forecasting relative loss rate

In summary, in mutagenic experiments with maize endosperm markers: (a) there is probably almost no selection, (b) nearly all marker losses are chromosomal, (c) the underlying chromosome behavior is sufficiently understood, (d) rate of breakage is about proportional to pachytene length. From this it follows that an approximate forecast of loss rate should be possible for any marker whose cytological position is known.

It goes without saying that no forecast can deal with differences in sensitivity between different strains, or in different environments, or with possible defects of dosimetry. Only a *relative* forecast can be attempted, dealing with ratios of sensitivity of different markers, measured simultaneously in the same experiment, and never with the absolute rate.

The approximate nature of any such forecast ought to be made clear at the outset. No claim is made that the theory is complete: on the contrary, it is a deliberate simplification, and much of the basic data are also subject to large errors. The aim is only to examine whether the production of so-called mutations in maize is intrinsically a predictable process in which respect it would differ radically from superficially similar experiments in such organisms as Drosophila or mice. The possibility of making such a forecast in maize was pointed out in a note (FABERGÉ 1956b).

REVIEW OF LITERATURE

Data on induced marker loss in maize

Extensive data on rates of loss of endosperm markers have been published by STADLER and UBER (1942) using ultraviolet radiation, and by KONZAK and SINGLETON (1956a, b) using X-rays, thermal neutrons, and ultraviolet. Some additional data can be extracted from three earlier papers by STADLER and SPRAGUE (1931a, b, c). In all of these several markers were used simultaneously, so that comparisons are always possible within the same batch of material and from the same treatment. Fortunately the marker *Pr* happens to have been used on every occasion, thus providing a convenient point of reference among different experiments; for this reason, we shall express relative sensitivity with respect to $Pr = 1$. Clearly, an exact comparison of sensitivities is only possible if the dosage-effect function is exactly known, which in practice is not the case. This is another aspect of the difficulty discussed by STADLER and UBER (1942) in connection with the establishment of an ultraviolet action spectrum. Empirical information about the dosage-effect relation is statistically

insufficient, and some theoretical assumptions are always necessary for comparing sensitivities. While for X-rays, a satisfactory dosage-effect relation might conceivably be constructed on the basis of *a priori* theory, that is obviously not true of ultraviolet. Ultraviolet dosage-effect curves show a characteristic flattening at higher doses, more pronounced for the less penetrating wavelengths. A large part at least of this flattening can be shown to be caused by heterogeneity of the statistical population with respect to effective dose, for the flattening is greatly reduced by irradiating pollen from two sides instead of one (STADLER and UBER 1942). The complexity of the data makes it very difficult to attempt any kind of weighting by means of empirical dosage effect curves. For the immediate purposes of this paper, a much simpler practical procedure seems adequate, consisting of simple averaging. Effectively, the only weight is then the number of losses. This amounts to assuming that the *differences* between dosage-effect curves for various markers are themselves linear functions of the dose. This is most unlikely to be exactly true and is definitely untrue on the theory examined in a later section. However, when it is considered that the range of doses used in practice is quite restricted, being severely limited by insufficient effect at one end and killing at the other, such a simplified procedure will be seen to be a justifiable approximation. There are many other sources of error in the data which would make any refined weighting out of place. For instance, it seems likely that B.-F.-B. cycles may at times have been confused with fractional losses; in general, neither STADLER and UBER nor KONZAK and SINGLETON intended to include these in their data. However, the proportion of fractional losses to whole kernels varies strongly at times, suggesting the possibility of such confusion. At the time when some of the work was done, the B.-F.-B. cycle was not recognized as such. Because of the possibility of such errors, all data have been examined separately for entire kernel and for fractional losses In general, both give almost identical relative sensitivities for the various markers. An exception is Sh_1, which is ill suited for the detection of fractional losses.

The data of STADLER and SPRAGUE (1936a, b, c) and of STADLER and UBER (1942) are summarized in table 1. Entire and fractional losses have been added. Data from the last paper have been arbitrarily divided into three approximately equal sections for presentation, according to the purpose for which they were accumulated. It can be seen that there is rough agreement between them, leaving no doubt that the sensitivity of the markers is consistently in the order *A-Pr-Su*. It is obvious that the 12 individual entries will fluctuate much more on account of the smaller numbers; however, all but one of them agree in giving the same sensitivity order, and the one exception is no more than would be expected by random sampling with numbers of that magnitude.

Among the markers used by KONZAK and SINGLETON (1956a, b) are the 9S genes *Sh* and *Wx*. Since the constants used in the forecast are obtained in part from the relation between *Sh* and *Wx* in our own data, other data involving this same relation cannot be used for comparing with the forecast, as a fit is then inevitable. But some one of these markers can be used for comparing its sensitivity with other markers, in other chromosomes. With the combination in KONZAK and SINGLETON'S experiments, search for L(*Wx*) had to be made on colored kernels, a procedure which they recognized to be inefficient, and which in fact prevented them from including this class in their dosage effect curves. For the same reasons, we shall disregard L(*Wx*)

TABLE 1

Data on induced marker loss by STADLER *and coworkers*

	Total seeds examined	Losses both entire and fractional			Sensitivity, with $Pr = 1$	
		L(A_1)	L(Su)	L(Pr)	A_1	Su
STADLER and SPRAGUE (1936a,b, c) mainly filtered UV, some X-ray	13013	805	—	509	1.58	—
STADLER and UBER (1942) all UV. Dosage-effect relation at:						
5 doses, λ 2537	2383	66	34	71		
7 doses, λ 2650	6750	207	96	98		
4 doses, λ 2967 a	2054	135	60	95	1.76	0.77
9 doses, λ 2967 b	4854	367	166	164		
4 doses, λ 3022	2305	174	60	112		
Various conditions of pollination and treatment, all at λ 2537	2118	223	59	154		
	1212	113	55	44		
	1864	164	60	186	1.34	0.47
	1916	196	66	132		
	1789	132	50	102		
Action spectrum data 18 λ, several doses	13796	742	368	407	1.82	0.99
General total	54059	3324	1074	2074		

and use L(Sh_1). Sh_1 is not an ideal marker, its expression depending much on background; some entire L(Sh_1) may be missed, on the other hand, some other dominant effects may be mistaken for L(Sh_1). Diagnosis is more reliable if kernels are cut in half, but this does not seem to have been done in collecting these data. Fractional L(Sh_1) presents greater difficulties: in actual fact, KONZAK and SINGLETON list very few of them, much less than the proportion of fractional kernels for other markers. To compare the sensitivity of Sh_1 with that of other markers, we have the choice of either limiting comparison to the entire class only, or else correcting L(Sh_1) for the assumed proportion of fractional losses missed. We have preferred the former: the data given in the first part of table 2 therefore include only whole kernel losses, and are in fact identical with the numbers used by KONZAK and SINGLETON for their dosage effect curves. An excess of fractional L(R) is recorded, perhaps due to confusion with B.-F.-B. cycles; otherwise, relative sensitivity is the same among both entire and fractional classes.

In the case of KONZAK and SINGLETON (1956b), dealing with A_1 and Pr, we have followed the same procedure as with STADLER's data and have added entire and fractional classes. In this paper KONZAK and SINGLETON deal also with Sh_2, which is so closely linked to A_1, that these two markers are nearly always lost together. Although simultaneous loss of closely linked markers supports the general argument, it cannot be legitimately used as a test of forecasting, as will be seen later. For this reason, A_1 alone is recorded in the table. Independent loss is occasionally possible, more commonly from ultraviolet treatment.

It will be seen from the column headed relative sensitivity of table 2 that the three kinds of treatment give roughly concordant relative loss rates for the five markers, leaving no doubt about the order of sensitivity in which they would be

TABLE 2

Data on induced marker loss by KONZAK *and* SINGLETON

	Total seeds examined	Observed losses					Relative sensitivity with $Pr = 1$			
		L(A_1)	L(Pr)	L(R)	L(Su)	L(Sh_1)	A_1	R	Su	Sh_1
KONZAK & SINGLETON, (1956a) (entire losses only)										
Thermal neutrons, 5 doses										
1	15078	—	111	101	61	35				
2	9266	—	134	125	100	51				
3	7833	—	227	182	141	105	—	0.98	0.78	0.39
4	11622	—	434	405	372	172				
5	15181	—	625	692	530	323				
X-rays, 3 doses										
1	17054	—	219	196	168	91				
2	16223	—	417	359	354	163	—	0.92	0.83	0.44
3	13205	—	430	429	368	216				
UV λ 2537, 3 doses										
1	3996	—	56	39	20	21				
2	2830	—	62	61	39	24	—	0.81	0.56	0.38
3	1980	—	63	46	42	23				
KONZAK & SINGLETON (1956b)										
One dose each:										
Thermal neutrons	14427	841	537	—	—	—	1.57	—	—	—
X-rays	12141	633	347	—	—	—	1.82	—	—	—
UV	14903	1142	844	—	—	—	1.35	—	—	—
Total	155739									

arranged. Moreover, the relative sensitivities agree quite closely with those of STADLER's data, given in table 1. The only discrepancy is the sensitivity of 0.81 for L(R) in the ultraviolet data of KONZAK and SINGLETON; this, however, is based on much smaller numbers than the others, and is well within the range of statistical uncertainty. A general summary of all the data is given in table 5 which makes the general agreement obvious. It must be considered that the data were collected by different workers at different times, using different genetic stocks; that the mutagens include agents as physically different as X-rays, thermal neutrons and ultraviolet radiation of many wavelengths, and that different doses are included. In view of this background, the degree of agreement is remarkable. This diversity of sources of data and of treatments and the general agreement of the constituent parts, gives a very wide inductive basis for our conclusion: that the order of sensitivity of the markers is

$$A_1—Pr—R—Su—Sh_1$$

the highest, A_1 being about 3.8 times as sensitive as the lowest, Sh_1.

Location of endosperm markers

A number of maps of pachytene chromosomes of maize have been published. We have here used the most recent information and constructed the map in figure 1 on

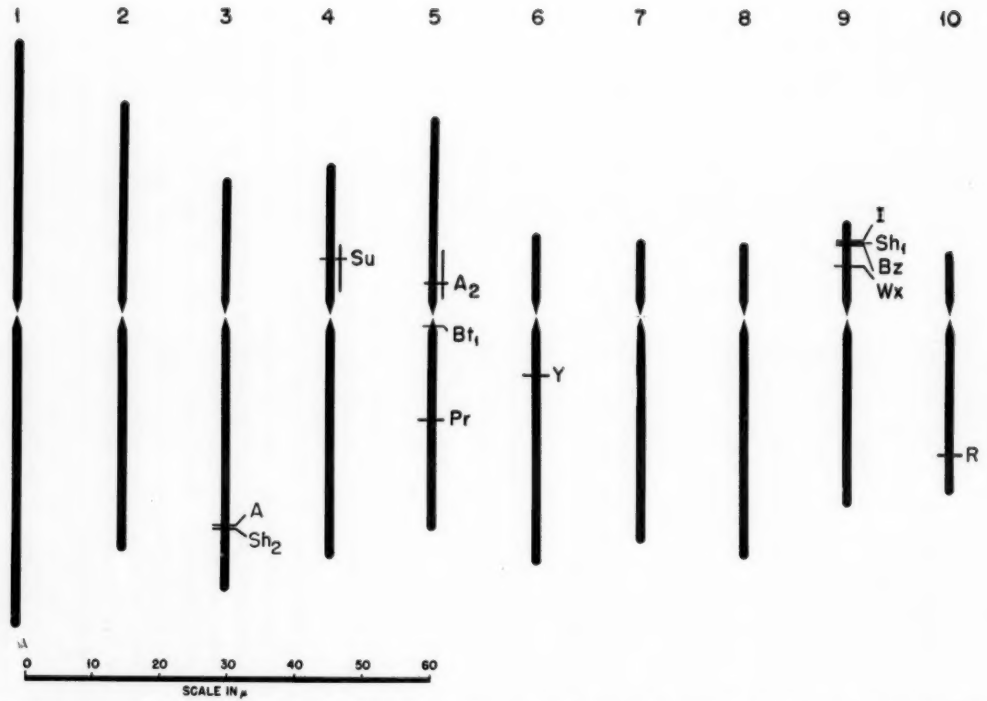


FIGURE 1.—Pachytene map of maize from the data of DO AMARAL GURGEL (1956). Principal endosperm markers located from data discussed in text. All positions are subject to uncertainty. The diagram is intended only as a representation of locations assumed in this paper, and is not to be taken as a standard map. Correction: "*A*" on chromosome 3 should read "A_1".

TABLE 3

Values of m

	Pooled data	X-ray data	UV data
Model assuming no stable terminal deletions	3.17	2.50	4.05
Model with stable terminal deletions	3.24	2.24	4.67

measurements by DO AMARAL GURGEL (1956) on strain KYS; our thanks are due to DR. GURGEL for permission to publish this. Discrepancies among different maps are by no means entirely due to errors of observation. Maize pachytene chromosomes stretch during spreading, and this is likely to be influenced by the presence of knobs which varies greatly between different strains. Thus, some of these discrepancies are doubtless real.

The location of markers on the cytological map of maize is rarely very precise. There are few detailed cytological landmarks, and length measurements are often difficult. Some markers, such as A_1, or those in 9S, are placed with considerable accuracy; the same is true for instance of *V*, as a number of translocations have break points close to that locus. In some other cases, where there is much genetic exchange between marker and translocation point, uncertainty can be considerable. In many instances, the inaccuracy of cytological location is a major source of error in the calculations. The information which has served for choosing the locations used in this paper is discussed below. These values are represented on the map in figure 1 and are given as p and d distances, in micra, in table 4.

1. A_1—Sh_2.—The position of A_1 in 3L is rather accurately known, the basic evidence being given in RHOADES and DEMPSEY (1953). We thank DR. RHOADES for giving some further information by letter. The best estimate seems to be 0.78 of 3L. Sh_2 is distal to A_1, and about 0.27 crossover units away from it (MAINS 1949; LAUGHNAN 1952). These two markers are thus so close that they are nearly always lost simultaneously, though rare separate loss is possible.

2. *Su*.—Of all the markers dealt with here, the position of *Su* is perhaps the least well known. Ambiguity arises from suppression of crossing over in the region. ANDERSON, KRAMER, and LONGLEY (1955a) give extensive data on translocations involving 4S, on the basis of which the position is assigned. A range of uncertainty has been indicated in this case.

3. Bt_1.—RHOADES (1936) and MCCLINTOCK (1938b) have shown that Bt_1 is very close to the centromere in 5L. This marker shows only about two percent exchange with Bm_1, which lies on the other side of the centromere. Of all known endosperm markers, Bt_1 occupies a position closest to a centromere.

4. A_2.—The information given by RHOADES (1936) places A_2 a short distance out on 5S, about seven crossover units from Bt_1; MCCLINTOCK (1938b) described a ring, one break of which was at 0.14 of 5S, which did not include the locus of A_2. The position given must be regarded as quite tentative; there may be crossover reduction in the centromere region.

5. *Pr*.—MORGAN (1950) has given an analysis of an inversion one break of which is almost exactly in the middle of 5L, and *Pr* can be located relative to this. Since

Pr occurs in all of the data on induced loss, and is convenient to use as a reference standard, more data on its exact locus is to be desired.

6. *Y*.—The position of *Y* seems to be quite accurately determined from the extensive data of ANDERSON, KRAMER, and LONGLEY (1955b) and the earlier ones of BURNHAM (1950).

7. *I-C*, Sh_1, *Bz*, *Wx*.—The positions of these markers are all rather reliably known, thanks to the work of MCCLINTOCK. The distances used here were those of RHOADES and PATTERSON (unpublished) which were also taken for analysis in FABERGÉ (1956a).

8. *R*.—Extensive evidence places *R* toward the end of 10L. In the normal 10th chromosome, *R* lies proximal to the distal one sixth of the arm, and shows about one percent recombination with this distal portion, which is distinguishable in special heterozygotes (RHOADES 1952). A recent paper dealing with translocations involving *R* is by ANDERSON and KRAMER (1954).

CALCULATION OF A FORECAST

General considerations

For the reasons given earlier it appears that the overwhelming majority of marker losses can be taken to consist of only two classes: proximal B.-F.-B. cycles and segmental deficiencies. In attempting to make an approximate forecast, we shall make the formal assumption that these two classes are sufficient. The general principles of estimating the necessary constants and the data on which they are based are discussed in FABERGÉ (1956a) so that only a brief review will be given here.

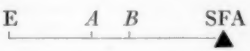
Breakage-fusion-bridge cycles

The contribution of proximal B.-F.-B. cycles is the simplest to deal with. In a multiple marker experiment, the frequency of distal B.-F.-B. is given directly by the data. Thus (FABERGÉ 1956a) we have 384 observed B.-F.-B. cycles between the end of the arm and *Wx* among 15042 kernels, from all treated material. Chiefly for statistical reasons, it has seemed slightly preferable to use as starting point the datum of 867 B.-F.-B. cycles in the entire 9S, a distance of about 14.2 micra, the assumptions made in estimating the B.-F.-B. cycles in the unmarked region are in fact exactly the same as in the forecast. In practice, either procedure leads to about the same result. We multiply this by the ratio of all visible B.-F.-B. cycles among X-ray treatments to B.-F.-B. cycles in all treatments, $867 \times 291/483 = 522$ estimated B.-F.-B. cycles among 6782 kernels resulting from treatment with 1500r of X-rays. Hence, $522/(14.2 \times 6782) = 0.00542$ B.-F.-B. cycles are induced per micron of cytological length by 1500r. To get the contribution of proximal B.-F.-B. cycles to losses at 1500r, we simply multiply the proximal pachytene distance in micra by 0.00542.

Segmental deficiencies

The estimation of the deficiency contribution requires a slightly more complicated argument. Segmental deficiencies are assumed to be generated by two distinct events or breaks, each of them occurring at a frequency proportional to cytological length.

Whether these two events are truly independent in a statistical sense would not in general affect this proportionality to length, which is the important point in the present context. Lack of independence would affect, for instance, the dosage-effect relation. The justification for this assumption is that it will account in a satisfactory manner for the relative frequency of deficiencies of different sizes in a multiple point experiment. A means of estimating such *deficiency generating events* is discussed in FABERGÉ (1956a). This estimate is a minimum, inasmuch as breaks which eventually restitute are not included: the calculation in effect assumes that whenever two such deficiency generating events or breaks occur in the same arm, the segment between them is invariably lost. It is not implied that Df generating breaks are physically in any way different from other breaks; they are merely a statistical class, distinguishable by their consequences. Both Cy generating breaks and Df generating breaks being taken as proportional to length, we represent their ratio, Df/Cy as m. If, relative to a marker, there are D distal Cy events and P proximal Cy events, there will also be mD and mP Df events. Of all cases when there are two Df events in that arm, $(mD)^2$ will have both events in the distal, $(mP)^2$ in the proximal segment, and $2m^2DP$ will have one event in the distal, one in the proximal segment. This last term is then the frequency of segmental deficiencies for the marker, in the other two cases the deficiency being unobservable. It has been seen that D and P, for 1500r, are obtained simply by multiplying pachytene length in micra by 0.00542; the only other information needed is m, which is obtained from multiple point data, by the following consideration. Let a chromosome arm carrying two dominant markers A and B be:



E stands for the natural stable end of the arm, and SFA for the spindle fiber attachment. From such an arm, five classes of affected offspring are recognizable. In an actual experiment, these observed frequencies will be samples, subject to statistical error, of the underlying population parameters. In the symbols proposed earlier:

Observed class	Corresponding parameter
Cy(EA)	α
Cy(AB)	β
L(A)	γ
L(B)	δ
L(AB)	ϵ

The class Cy (B SFA) = x is not separately recognizable. The assumptions made about the generation of deficiencies can be stated as three equations:

$$(1)\ \gamma = \alpha\beta K$$
$$(2)\ \delta = \beta K x$$
$$(3)\ \epsilon = \alpha K x + x$$

with only two unknowns, x and the constant K; it will be seen that $K = 2m^2$. It will be noticed that a constraint is implied by the equations, which can be expressed as:

$$(4)\ \epsilon = \frac{\alpha\delta}{\beta} + \frac{\alpha\delta}{\gamma}$$

There being only two unknowns, solutions for x and K can be obtained from any three pairs of equations (1) (2), (1) (3), (2) (3), if observed frequencies are substituted for the corresponding parameters. The three solutions will not be identical, however, unless the observations happen to obey relation (4) exactly. In the case of actual data, the three solutions differ but little. The correct procedure is to make a Maximum Likelihood estimation of the parameters, using simply the relation:

$$\alpha + \beta + \gamma + \delta + \epsilon - 1 = 0$$

and substituting (4) for ϵ, to get the Log Likelihood equation. Expressions are then obtained for

$$\frac{\partial L}{\partial \alpha}, \quad \frac{\partial L}{\partial \delta}, \quad \frac{\partial L}{\partial \beta}, \quad \frac{\partial L}{\partial \gamma};$$

which, equated to 0, yield two linear and two quadratic equations. These are solved by iteration. I am much indebted to DR. M. KASTENBAUM for pointing out that the Maximum Likelihood equations may be arrived at in a simpler manner by the application of Lagrange multipliers to the Log Likelihood function.

Applying this method to the pooled data of FABERGÉ (1956a), $K = 20.156$, hence $m = 3.17$.

At this point a modified hypothesis for the origin of deficiencies can be considered. We might suppose that in addition to the two contributing classes of proximal B.-F.-B. cycles and segmental deficiencies, there is a third class of stable terminal deletions. Assuming that these also appear in proportion to the length scale provided by Cy events, a scale which, incidentally, need not correspond to physical length to make the method valid, we represent this frequency as T Cy. T can now be introduced as a third unknown into (1) (2) and (3), yielding:

$$\gamma = \beta(\alpha K' + T)$$
$$\delta = \beta K' x'$$
$$\epsilon = (\alpha K' + T + 1)x'$$

These are solved directly, no estimation being required. $T(= -0.038)$ is a small negative number, not different from 0 statistically. On this basis, it was concluded (FABERGÉ 1956a) that stable terminal deficiencies either do not occur at all or only at a low frequency. For our present purpose, we also note that $K' = 21.02$ and $m' = 3.24$, values so close to K and m that they would give essentially the same result if used in the forecast calculation.

The value of K (and m) is estimated from pooled data, including both X-ray and ultraviolet treatments. There is no assurance that these two treatments necessarily yield the same K. It may correspond to a fundamental property of chromosomes and be independent of the nature of the mutagen, or it may not. With the data available, sampling errors become rather large when m is estimated separately from ultraviolet and from X-ray data. The results of this calculation are given in table 3; we are much indebted by DR. M. KASTENBAUM for help in connection with them. While m for X-rays is about 1.8 times greater than for ultraviolet, it seems unlikely that this difference is significant. For the present purpose we have therefore pre-

ferred to take $m = 3.17$ from pooled data. More extensive experiments may of course give occasion to revise this. There are so far no data from which m could be calculated for neutrons, nor can it be verified that it is independent of dose.

It is worth noting in this connection that separate calculations for the two radiations lend no support to the idea that ultraviolet generates true stable terminal deficiencies in maize endosperm. In no case does T differ significantly from 0, but it is negative for ultraviolet data (-0.279) and positive for X-ray data ($+0.124$).

Estimate for total losses

The forecast is made simply as:

$$L = 0.00542p + (0.00542)^2Kpd$$

where L is the loss rate, p and d the proximal and distal pachytene lengths in micra. The results are presented in table 4. For the sake of completeness, this table includes all of the convenient endosperm markers, including those for which no data exist. The 9S markers *I*, *Sh*$_1$, *Bz* and *Wx* from which the constants used in the forecast were estimated are also included. These last form no part of the comparison; in their case, a close fit is assured. Since the comparison of forecast with observation is being made on a purely relative basis, arbitrarily taking $Pr = 1$, some one of the four markers of 9S may be legitimately included, since nothing is implied about the relation of its sensitivity to that of markers located elsewhere than in 9S. For the reasons given in the section dealing with data, *Sh*$_1$ is chosen as this one marker.

For convenient comparison, summaries of forecast and observation are presented side by side in table 5, the loss rates being given relative to $Pr = 1$ as in table 4.

While discrepancies are obvious, it can be seen that the forecast places these five markers in the same order of sensitivity as the observations. So many approximations have been made that it does not seem worth while to discuss these discrepancies

TABLE 4

Tentative forecast calculation

Marker	Most likely pachytene distances (μ)		Distal B.-F.-B. cycles	Proximal B.-F.-B. cycles	Interstitial deletions	Estimated loss rate at 1500r
	p	d				
Sh$_2$ *A*$_1$	31.4	8.8	0.048	0.170	0.164	0.334
Su	6.7	15.6	0.085	0.036	0.062	0.098
Bl$_1$	1.6	29.9	0.162	0.009	0.028	0.037
A$_2$	5.0	24.0	0.130	0.027	0.071	0.098
Pr	14.5	17.0	0.092	0.079	0.146	0.225
Y	6.2	30.3	0.164	0.034	0.111	0.145
Wx	7.1	7.1	0.039	0.039	0.030	0.069
Bz	10.8	3.4	0.018	0.058	0.022	0.080
Sh$_1$	11.1	3.1	0.017	0.060	0.020	0.080
I-C	11.7	2.6	0.014	0.063	0.018	0.081
R	21.2	4.8	0.026	0.115	0.060	0.175

TABLE 5

Comparison of forecast with observation

Marker	Forecast	Observed			
		Stadler and Sprague (1936a, b, c)	Stadler and Uber (1942)	Konzak and Singleton (1956a)	Konzak and Singleton (1956b)
Sh$_1$	0.36	—	—	0.41	—
Su	0.44	—	0.69	0.79	—
R	0.78	—	—	0.95	—
Pr	1.00	1.00	1.00	1.00	1.00
A_1	1.48	1.58	1.61	—	1.51

All loss rates arbitrarily expressed relative to *Pr* = 1.

It will be noted that a closer fit is obtained if all forecast estimates are multiplied by an arbitrary factor of about 1.2.

in detail. The choice of *Pr* as common standard was made solely because it happens to have been used by all the workers; it is otherwise quite arbitrary and its chromosome location is not as accurate as might be desired. As expressed in table 5, all forecasts other than *Pr* are lower than expectation. It will be seen that a closer fit is obtained by multiplying all forecasts by an arbitrary figure of about 1.2 which has not been the subject of any statistical estimation. It is obvious that the estimate for *Pr* may easily be in error by such an amount from inaccuracy of cytological placement alone. It has seemed preferable, however, to present the observations and the forecast side by side without any fitting whatsoever, as otherwise judgment as to the reality of the forecast becomes subjectively loaded. These discussions only apply to endosperm losses which result from treatment of ripe pollen, and would not be expected to hold for chronic irradiation experiments in which there are intervening mitoses.

Dosage-effect relation

No multiple factor data with several doses exist which would permit a detailed analysis. In view of these, a few remarks seem justified about the consequences of the chromosome mechanism here proposed on the expected dosage-effect relation for simple loss. The first step in the forecast calculation was multiplication of cytological length, in micra, by the factor 0.00542, which is the number of B.-F.-B. cycles per micron at 1500r of X-rays. The most obvious means of extrapolation to another dose would be to use as a factor 0.00542 × dose/1500, the calculation beyond this point being carried out exactly as before. There is no empirical information at to whether the factor *m* is independent of dose, and no theoretical considerations give any guidance. Making the simplest assumption, that *m* is in fact independent of dose, expected dosage effect curves are given in figure 2. Presumably it is not the probability, *n*, of inducing a B.-F.-B. cycle generating event which is needed, but the probability of inducing *one or more* such breaks in the given segment. Supposing these events to be independent, which is quite likely to be true for X-rays, the sum of the Poisson distribution terms other than the first is needed, $1 - e^{-n}$.

This correction becomes appreciable for the more sensitive markers involving

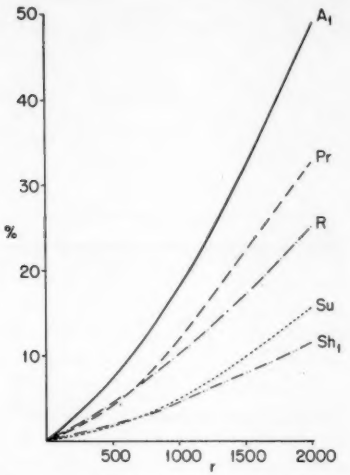


FIGURE 2.—Dosage-effect curves expected for X-rays on the model assumed for the generation of marker losses.

longer chromosome segments at the higher doses, and has been included in the calculation. It is obvious, however, that much greater errors than this correction are made in the approximate assumptions.

The dosage effect curves based on these simple assumptions are mixtures of linear and quadratic components, superimposed on which is a depression due to coincidence, or the Poisson distribution correction just mentioned. It is clear that at doses used in practice, the *relative* sensitivity of markers will not change much with dose. It is hoped that the procedure used in summarizing the data, consisting of simple addition, is justified for a reason of this kind. At very low doses there are instances of markers cutting one another, a fact which it would probably be impractical to verify at these low rates. It is obvious that the general shape of the curves published by KONZAK and SINGLETON (1956a) does not contradict these approximate expectations. The absolute rates of loss reported by these authors is, however, much less than in our own data at the same X-ray dose.

A different form of calculation should be considered for densely ionizing particles, when it might be expected that one primary particle is usually responsible for the two breaks of a deficiency.

Effect of the position of a marker on its sensitivity

The model used implies that the sensitivity of a marker depends both on the absolute length of the arm and on its position in that arm. This model can be said to be essentially verified for 9S, among the shortest in maize, and in making the proposed forecast, we have extrapolated it to all arms, including long ones like that carrying A_1. Just as in the case where dose is the variable, there is no direct verification that m will be the same in arms of all length, or in different parts of a long arm. Making

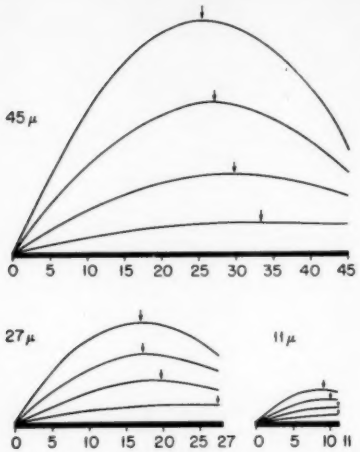


FIGURE 3.—Relation expected, at various X-ray doses, between the sensitivity of a marker and its position on a chromosome arm.

this simplifying assumption, the expected effect of displacing a marker along an arm is plotted in figure 3. The coincidence correction, $1 - e^{-n}$, at time fairly large, has been applied. The calculation is made for the longest (45 μ) and the shortest (11 μ) arm of the maize complement, as well as for one of intermediate length (27 μ). The position of maximum sensitivity is marked with an arrow. It will be seen that for a short arm, maximum sensitivity is expected to be at the end, but for a long one, at the higher doses, is very nearly in the middle. The expected reduction of sensitivity as one goes from the middle of a long arm towards its distal end might lend itself to experimental verification.

The possible use of induced loss rates to locate markers

The question might be asked whether the forecast procedure might not be used in reverse, so that a marker locus could be deduced from the loss rate.

The data of FABERGÉ (1956a) show that the points of induction of B.-F.-B. cycles could perhaps be so used, as they are simply and directly related to length. The markers must be of such a kind that these B.-F.-B. cycles can be identified with certainty, and generally more than one must be present in an arm, to provide a reference scale independent of sensitivity or dose. These conditions will be rarely met in practice, though it is worth pointing out that the method is, in principle, a means of genetic mapping entirely independent of crossing over or of any meiotic phenomena whatsoever.

The use of simple loss data for this purpose seems very questionable except in the special case of markers very close to one another. For a single independent marker, a quadratic must be solved even if the constants can be taken as known. Taking for example the constants for 1500r used here and making L = the observed loss rate,

A = the total length of the arm in micra, and and p = the unknown proximal distance in micra, we would have:

$$-0.000592p^2 + (0.000592A + 0.00542)p - L = 0.$$

It does not seem that this relation will prove of practical use.

DISCUSSION

Test of reality of forecast

It is worthwhile to consider briefly the general principles involved in testing whether such a forecast is real. The problem is logically rather different from the familiar statistical tests used in examining data. Fundamentally, the success of any forecast whatsoever is assessed by comparison with another expectation obtained without the forecast, from some null hypothesis. The comparison can then be a statement of the probability of getting as good or better forecasting by chance. In testing fit with a Mendelian expectation, for instance, the theory giving this expectation is taken to be complete, and the null hypothesis is rather easily and precisely specified.

It is not claimed that the forecast used here is based on a complete theory: a great many rough approximations and simplifications were made, and factors known to be operating have been deliberately disregarded as probably unimportant. We do not, consequently, expect a fit of the kind considered in examining Mendelian segregations. We are concerned with testing only whether the forecast is real.

An appropriate null hypothesis is not easy to specify. At first sight we might consider the simple hypothesis of *complete ignorance*: that nothing whatever is known in advance about induced loss rate in maize. This is not, however, the hypothesis that it is desired to test; if nothing whatever is known about loss rates, then the mere prediction that loss occurs at all has to be considered a significant success.

In actual fact, there is a great deal of *a priori* information, and we must begin by defining this. Not only is loss known to occur, but it is also known to be extremely high compared with other organisms. For a maize endosperm marker, ten percent loss might be induced by 2000r of X-rays, a rate per roentgen of 5×10^{-5}, which is about 1000 times that for the relatively sensitive locus garnet in *Drosophila melanogaster* (VALENCIA and MULLER 1949), or about 50 times that for *S* in mice (RUSSELL 1951). These high rates in maize, high not only relative to physical dose, but absolutely, are presumably largely the expression of absence of selection in this material. However, no *a priori* knowledge gives any guidance as to the *relative* sensitivity of different markers, with the exception of closely linked loci. Here the mere knowledge that the mechanism of loss is chromosomal leads one to expect closely linked markers to be often lost simultaneously. We shall therefore disregard the fact that this expectation is verified for A_1 and Sh_2, in assessing the goodness of our forecast. With this exception, no previous experience in other organisms provides any basis for expectation of ratios among mutation or loss rates for different markers. Among the loci studied by ALEXANDER (1954) in *Drosophila melanogaster*, the most sensitive differed from the least sensitive by a factor of about 4.4, but in unpublished data of PATTERSON quoted by her, a ratio of 22 is found. Among the rates reported by

VALENCIA and MULLER (1949), garnet differed from raspberry, scute, or achaete by a factor of about 30. In mice, RUSSELL (1951) reports a difference in sensitivity of the order of 30-fold among his markers. We are here disregarding cases of genes which have never been observed to mutate in experiments, or special situations such as *y* in Sc^8, where the position of the locus increases its apparent loss or inactivation rate by a factor of about 20.

With this background, a simple nonparametric test suggests itself, which can be used as a partial basis for the assessment of the forecast. It has been shown in the section dealing with empirical data, that the observed order of sensitivity is, without any ambiguity,

$$A_1—Pr—R—Su—Sh_1$$

A_1 being the most sensitive. We can make the null hypothesis that the order of sensitivity is unknown *a priori*, and that any order is as likely as any other, without saying anything at all about how much one marker differs from another. As was discussed in the section dealing with the forecast calculation, only one of the markers of 9S can be used in such a comparison, and Sh_1 was chosen as that one marker because it is adequately represented in the data of KONZAK and SINGLETON (1956a). Five markers can be ordered in $5! = 120$ ways. Table 5 shows that the forecast gives this order, A_1, *Pr*, *R*, *Su*, Sh_1 correctly, a result that would occur at random once in 120 trials. It should particularly be noted that no simple extension of *a priori* information other than the proposed theory will yield this result: for instance, sensitivity is not merely a function of distance from the centromere, which would give the order A_1 *R Pr* Sh_1 *Su*.

It may be remarked that this simple order of sensitivity test is not universally applicable in all cases. For instance, table 4 shows that the very tentative expected sensitivities for *Su* and for A_2 are the same. In this instance, the forecast only tells that they would not be expected to differ a great deal from each other.

It will be obvious that a more powerful test could legitimately be used, if it were possible to formulate an appropriate null hypothesis, and that the simple nonparametric order test gives a bare minimum assessment of success. This test entirely disregards the ratios of the sensitivities of markers; it would be equally valid if A_1 turned out to be 1000 times more sensitive than Sh_1 and considers order only. In actual fact the forecast predicts these ratios with considerable success: the most sensitive marker, A_1, is found to be 3.8 times as frequently lost as the least sensitive, Sh_1, the prediction being 4.2 times. Such facts are difficult to fit into a formal statistical test. It seems safe to conclude nonetheless, that the probability of achieving a forecast as good or better than the one proposed, by some alternative random process is many times less than 1 in 120.

SUMMARY

1. The nature of single marker losses observed in maize endosperm, when pollen carrying dominants is irradiated, is discussed. It is pointed out that: a. there is little or no selection. b. losses are preponderantly chromosomal rather than genic. c. an approximate model of chromosome behavior following breakage can be deduced

from multiple point experiments. d. the rate of breakage is about proportional to pachytene length.

2. It follows from the preceding that enough information is available to attempt an approximate forecast of relative loss rate for any marker whose cytological position is known.

3. Using only constants estimated from markers in the short arm of chromosome 9 a tentative forecast is given for other endosperm markers located elsewhere.

4. Published data are analyzed to establish the observed relative loss rates for *Sh*$_1$, *Su*, *R*, *Pr*, and *A*$_1$.

5. It is shown that the forecast places these five markers in the correct order of sensitivity, and moreover gives their relation to one another about correctly.

6. It is concluded that the forecast is real and cannot, within reasonable limits of probability, be considered fortuitous.

7. The effects of the chromosome model considered on expected dosage effect curves, and on the influence of position in the chromosome arm on sensitivity are discussed.

ACKNOWLEDGEMENTS

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CYTOGENETIC STUDIES OF ZEA HYPERPLOID FOR A CHROMOSOME DERIVED FROM TRIPSACUM

MARJORIE P. MAGUIRE

Cornell University, Ithaca, New York

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INTERGENERIC hybrids of *Tripsacum dactyloides* and *Zea mays* were first produced and studied by MANGELSDORF and REEVES (1931). Since only unreduced female gametes of these first generation hybrids functioned, the first backcross to Zea gave 38 chromosome plants of the constitution ZZT, containing two Zea sets each with 10 chromosomes and one Tripsacum set with 18 chromosomes (MANGELSDORF and REEVES 1932). At meiosis in these 38 chromosome plants they reported apparently regular synapsis of the two sets of Zea chromosomes and only occasional synaptic association of Tripsacum and Zea chromosomes, so that gametes having a complete set of Zea chromosomes plus 0 to 18 Tripsacum chromosomes were produced. Since in subsequent backcrosses to Zea as the pollen parent extra Tripsacum chromosomes were transmitted to the progeny with low frequency, Zea plants hyperploid for various numbers of Tripsacum chromosomes were readily isolated for study (MANGELSDORF and REEVES 1939).

In previous work of the author (MAGUIRE 1952) seven stocks[1] were studied which had been isolated from Zea backcross progenies of *Zea mays-Tripsacum dactyloides* hybrids in the manner described above. These stocks carried in each case a normal Zea complement plus a single chromosome derived from the Tripsacum complement. Studies were made of synaptic behavior, chiasma formation and phenotypic effect of these Tripsacum-derived chromosomes.[2]

In these previous studies by the author one 21 chromosome stock was of particular interest. This was a third backcross to Zea in which the extra Tripsacum chromosome was found at pachytene to have a total length of about 42 percent of Zea chromosome 1, an arm ratio of about 3.8:1, and a terminal knob on its long arm. This Tripsacum chromosome was usually paired at pachytene with the distal portion of the short arm of Zea chromosome 2, often forming a Y-shaped trivalent configuration as illustrated in figure 1A and 1B. In these trivalents the knob-bearing end of the Tripsacum chromosome was always paired with the distal portion of the short arm of one of the Zea chromosomes 2 for about 23 percent of its total length. The other end of the Tripsacum chromosome, including its centromere, was paired with the corresponding portion of the short arm of the other Zea chromosome 2 in about half the configurations, and incompletely paired or totally unpaired in the remainder. Sporocytes from individual anthers showed all of these types of configura-

[1] Material supplied by DR. L. F. RANDOLPH.

[2] Although it is impossible to determine to what extent the Tripsacum-derived chromosomes may have been altered during the preceding hybrid generations when they occupied the same nucleus with Zea chromosomes, for the sake of simplicity they will be referred to hereafter as Tripsacum chromosomes.

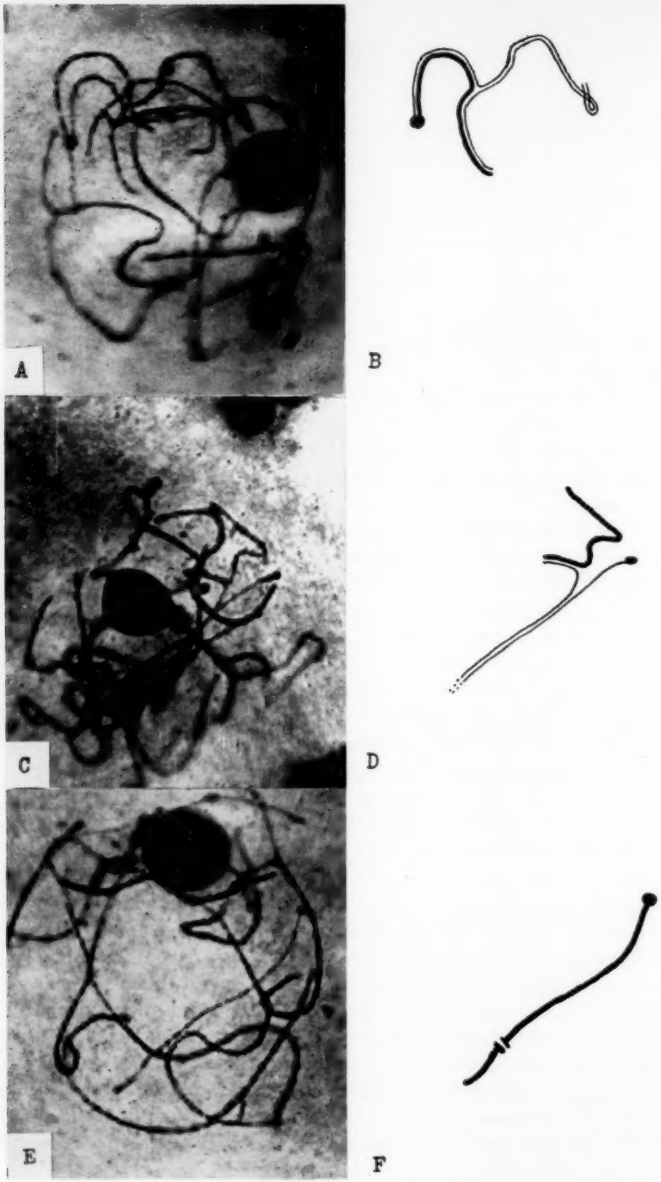

FIGURE 1.—Photomicrographs and semidiagrammatic camera lucida interpretations of univalents of the Tripsacum chromosome (E, F) and trivalents involving Zea chromosome 2 and the Tripsacum chromosome (A, B, C, D). Centromere positions, where detectable, are indicated by double cross lines. A heavy line denotes the Tripsacum chromosome in camera lucida interpretations of trivalent configurations. Figures C and E appeared among the sporocytes of a single plant. Magnification is about 1000 ×.

tion, and there were no apparent differences in pairing among several plants of this stock, although only one plant gave pachytene configurations sufficiently well spread for extensive analysis. Foldbacks were rare among the few univalent Tripsacum chromosomes found.

Although both ends of the Tripsacum chromosome paired with the distal portion of the short arm of Zea chromosome 2, only the knob-bearing end which was perfectly paired in all trivalents was interpreted as probably homologous to that segment of Zea chromosome 2. The irregular pairing involving the other end of the Tripsacum chromosome was probably non-homologous and comparable to the non-homologous pairing described by McCLINTOCK (1933) for other chromosomes of the Zea complement.

Trivalent configurations were found at pachytene in 90 cells from a total of 96 cells studied (about 94 percent). Since in all trivalents the knob end of the Tripsacum chromosome paired with the end of the short arm of Zea chromosome 2, and since random pairing among three homologues would explain only $\frac{2}{3}$ pairing for any homologous parts of two of them, clearly this is non random. Thus there appeared to be a tendency for the knob-bearing end of the Tripsacum chromosome to pair with a short region of one of the Zea chromosomes 2 to the exclusion of the other Zea chromosome 2, although these two Zea chromosomes apparently paired normally in the absence of the Tripsacum chromosome.

Another observation of interest in the original studies of this 2n plus 1 stock was cytological evidence of crossing over between Zea chromosome 2 and the Tripsacum chromosome. A disomic plant was found in which one of the Zea chromosomes 2 carried a terminal knob on its short arm (a previously unreported knob position in Zea), and several trisomic plants were found in which the Tripsacum chromosome was knobless. Metaphase I trivalent frequency was measured in several 2n plus 1 plants and found to be about 69 percent. Such a metaphase I chiasma frequency is somewhat higher than that expected from the normal amount of crossing over reported for disomic Zea in this region of chromosome 2 assuming approximately uniform distribution of crossover frequencies along the physical length of the chromosome. If such an assumption is made, only about 55 percent trivalents at metaphase would be expected, since about 23 percent of a chromosome carrying 128 crossover units (RHOADES 1955) was involved in pachytene trivalents with a frequency of about 94 percent. Thus the possibility was suggested that the crossover frequency was unexpectedly high between Zea chromosome 2 and this Tripsacum chromosome.

Phenotypically, the 2n plus 1 plants of this stock were not strikingly different from their disomic sibs, except for their pollen sterility. Plants carrying the Tripsacum chromosome were almost completely pollen sterile while those without it were normal.

The present work was undertaken in an attempt to gain information on the nature of the pachytene pairing described above, to determine knob recombination frequencies, metaphase I chiasma and genetical recombination frequencies for the region of Zea chromosome 2 which paired regularly with the Tripsacum chromosome, and to determine possible phenotypic effects of the Tripsacum chromosome.

Three stocks, each carrying a normal Zea complement plus the Tripsacum chromosome described above with its terminal knob, were pollinated in two successive gen-

TABLE 1

Transmission frequency of the Tripsacum chromosome

Stock no.	No. of 20-chrom. plants	No. of 21-chrom. plants	Percent transmission		
			Observed	Confidence interval	χ^2 for 50 percent transmission
P5401	34	33	49	41–59	.0075
P5402	50	25	33	22–44	8.30
P5403	38	27	42	32–52	1.86
Total P5401 P5403	72	60	45	38–52	1.09

erations by a Zea chromosome 2 genetic tester stock which was homozygous recessive for *lg*, *gl*, and *v*, and was known to give well spread pachytene preparations. Segregation ratios were recorded in the T_2 generation. Microsporocyte samples were collected and preserved in a 3:1 alcohol-acetic mixture under refrigeration, and acetocarmine smears were prepared from this material for microscopic study.

OBSERVATIONS AND DISCUSSION

Metaphase I studies: Transmission frequencies of the Tripsacum chromosome through the female gametes, based on metaphase I chromosome counts, are listed in table 1. Though the transmission frequency of this chromosome at least roughly approximated the maximum of 50 percent in stocks P5401 and P5403, it was markedly less in stock P5402. Since univalents were found in only five percent of microsporocytes at metaphase I in the female parent, and lagging chromosomes appeared in only two of 408 anaphase I cells and in only seven of 624 anaphase II cells, this lower transmission in stock P5402 appears not to have resulted from loss of the Tripsacum chromosome as a lagging univalent. Thus either the behavior of the Tripsacum chromosome in microsporocytes differed from that in megasporocytes, or there was some tendency for the Tripsacum chromosome to be included in the nonfunctional megaspores. Selective segregation has been noted previously in corn by LONGLEY (1945), MORGAN (1950) and RHOADES (1942, 1952) and in Drosophila by STURTEVANT (1936) and STURTEVANT and BEADLE (1936).

Metaphase I trivalent frequency (table 2) in 21-chromosome plants was found to vary significantly with presence or absence of the terminal knob of the Tripsacum chromosome; knob-bearing plants showed an average trivalent frequency of 90 percent, whereas knobless plants showed an average trivalent frequency of 69 percent. However, two of the 59 knob-bearing 21-chromosome plants showed trivalent frequancies very unlike the remaining 57 plants. The trivalent frequencies of these two exceptional plants were comparable to those of knobless 21-chromosome plants. Conversely, two knobless 21-chromosome plants showed metaphase trivalent frequencies within the range for knob-bearing 21-chromosome plants. These exceptional plants suggest the presence of genetic material influencing frequency of trivalent formation (or crossing over) located close to the knob, but separable from it by crossing over or by some other form of transfer of chromosome material. The

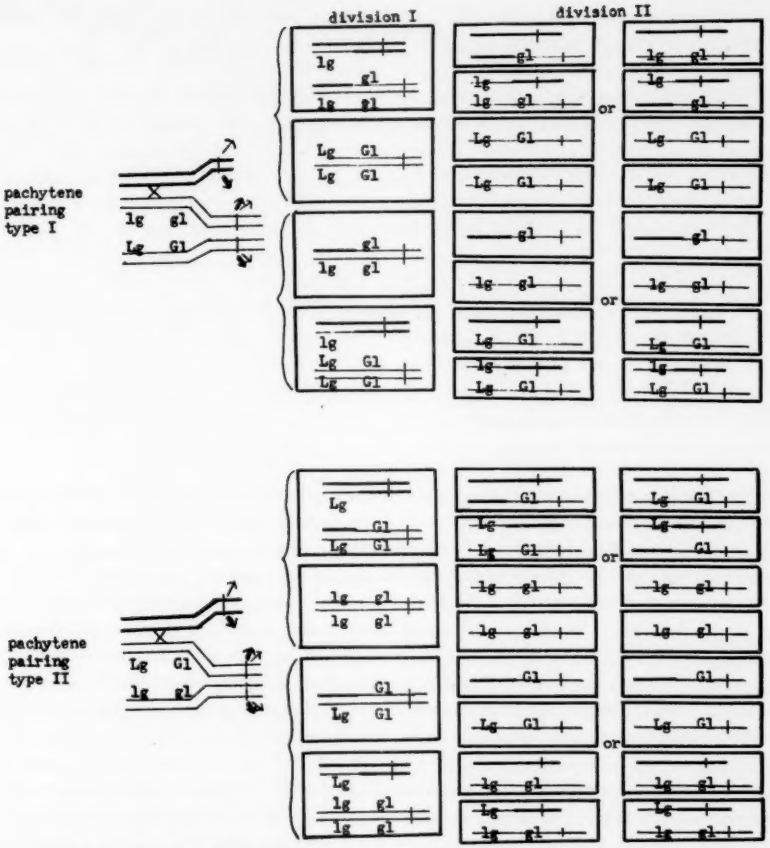

FIGURE 2.—Types of segregation into gametes from 21-chromosome plants following trivalent formation and crossing over in the *Lg—Gl* region. The Tripsacum chromosome is indicated by a heavy line; centromere positions are shown by vertical crosslines. No attempt has been made to represent relative lengths of the chromosome arms, and the location of the terminal knob is not indicated because of its instability. The lighter arrows indicate anaphase distribution type I, the heavier arrows anaphase distribution type II.

results indicate that at least in many of the plants (most of those bearing the knob and two without it) chiasma formation between the Tripsacum chromosome and Zea chromosome 2 usually followed their pairing. This is an extremely high chiasma frequency in view of the short region of Zea chromosome 2 involved; it will be discussed further in the section on genetic studies. Lacking knowledge of pachytene trivalent frequency it is impossible to say whether chiasma frequency was likewise a close reflection of pachytene pairing frequency in knobless plants.

TABLE 2

Metaphase I trivalent frequency in 21-chromosome plants

Stock no.	Knob	Trivalent frequency (%)											
		41–45	46–50	51–55	56–60	61–65	66–70	71–75	76–80	81–85	86–90	91–95	96–100
P5401	+						1			2	6	11	3
	−	1					6						
P5402	+						1			4	4	8	1
	−						1	2		1			
P5403	+										7	4	7
	−					1	2	1	1	1			
Total P5401, P5402, P5403	+						2			6	17	23	11
	−	1				1	9	3	1	2			

Determinations of metaphase I trivalent frequency are based on about 100 sporocytes from each plant. The difference between means of knob-bearing and non-knob-bearing plants is very significant (t test, one percent level).

Pachytene studies: Most plants were easily classified at pachytene for presence or absence of the terminal knob of the Tripsacum chromosome, and trivalent configurations similar to those observed in the previous studies were noted. In addition, a type of trivalent configuration (illustrated in Fig. 1C and 1D) in which the knob-bearing region was unpaired, also was frequently found. A possible explanation for this pairing difference will be discussed later. Since univalent Tripsacum chromosomes and trivalent configurations of this new type clearly indicate whether the knob is located on the Tripsacum chromosome or has been transferred to Zea chromosome 2, it was thought that a thorough study of available pachytene material would yield an estimate of the frequency of cytological recombination between these two chromosomes. However, as this study progressed, differences were observed among sporocytes of single plants and even single anthers as to whether the knob was on the Tripsacum or Zea chromosome. These observations suggested that some transfer mechanism other than normal meiotic crossing over must be involved. Examples of such knob transfers are illustrated in figure 1C, 1D, 1E, 1F. In two plants trivalent configurations were found in which the knob was located on one of the Zea chromosomes and absent from the Tripsacum chromosome, while univalent Tripsacum chromosomes in other sporocytes of the same plants carried the knob. Conversely in a trivalent configuration from a third plant the knob was on the Tripsacum chromosome and absent from Zea chromosome 2, while a univalent Tripsacum chromosome in another sporocyte of this same plant was knobless, the knob being on the Zea chromosome.

The mechanism involved in these apparent transfers of chromosome material is not at present understood. Since the knob transfers in all cases involved parts of chromosomes which could synapse regularly, and since they resulted in no apparent length change, the hypothesis is suggested that they resulted from some form of breakage and recombination when these chromosomes were intimately associated in

some premeiotic cell generation, essentially as they are during normal synapsis in the sporocyte. If the observed exchange occurred at zygotene or pachytene, many of the trivalent configurations (as in fig. 1C, 1D) would then require that a prediplotene opening without chiasma formation followed pairing, and either that the exchange occurred at a two strand stage or that all four chromatids were involved in each exchange, since two knob-bearing chromatids were always separated from two knobless chromatids in trivalents of the type illustrated in figure 1C, 1D. Such a meiotic exchange would differ from normal crossing over in time of occurrence or mechanism. If premeiotic transfer occurred, some mechanism similar to somatic crossing over in the sporogenous tissue may have been involved. It is not possible to say from the evidence at hand whether only the knob was transferred or whether there was an exchange of chromosome segments of greater length.

The material under study is unusually favorable for the observation of such instability of knob location because chromosome parts remain unpaired in some trivalents and because there is a pronounced difference in length between the Tripsacum and corn chromosomes. Thus it can be readily seen in these trivalents whether or not the knob is on the Tripsacum chromosome.

The cytologically observed shift in the position of the terminal knob from the Tripsacum chromosome to Zea chromosome 2 in different sporocytes and anthers of individual plants is comparable to the shifts in position of chromosome segments or genes postulated by McCLINTOCK (1950, 1951, 1952), BRINK and NILAN (1952) and BARCLAY and BRINK (1954) to explain certain characteristics of mutable loci, and to genetic transfer without crossing over (MITCHELL, 1955a, b). Further study of the problem is in progress.

An attempt to determine trivalent frequencies at pachytene was generally unsuccessful. With the exception of the sporocytes of a single plant whose pachytene configurations were unusually clear, it was not possible to classify more than one cell in 50 as to whether the extra chromosome was present as a univalent or in a trivalent association. Of the sporocytes from that single good pachytene plant it was possible to classify 124 cells from a total of 368. Of these 124 cells 119 or 96 percent contained the extra chromosome in trivalent association. Since the metaphase I trivalent frequency from this plant was 94 percent, the very high pachytene trivalent frequency was expected.

An attempt was also made to determine from the pachytene trivalents of this plant which regions of the chromosome if any were unpaired at this stage. Of 76 cells with clearly delimited trivalents which were examined for this purpose, the chromosomes were paired throughout in 20 trivalents; in 53 trivalents the Zea chromosome carried the terminal knob and was unpaired for some distance at the knob end, and the Tripsacum chromosome was likewise unpaired for a corresponding distance; in three trivalents the Zea chromosome carrying the knob was paired with the Tripsacum chromosome while the other Zea chromosome and the Tripsacum chromosome were unpaired for a considerable distance.

These results suggest an explanation for the unexpectedly high trivalent frequency consistently observed in knob-bearing plants. It is conceivable that in an earlier generation an exchange of material occurred between Zea chromosome 2 and the

Tripsacum chromosome involving segments which differed somewhat but were sufficiently homologous to pair in the absence of closer homologues (fig. 3). Thus in plants which received an altered Zea chromosome 2 and an altered Tripsacum chromosome from the female parent and a normal Zea chromosome 2 from the male parent, the mode of pairing for this region that would bring together those parts most alike, would involve the altered Tripsacum chromosome with the normal Zea chromosome. The altered Zea chromosome bearing the knob end of the Tripsacum chromosome would then be expected usually to remain unpaired at this end, the usual condition observed in the plant described above. Accordingly, in the absence of the Tripsacum chromosome the altered and normal Zea chromosomes 2 paired regularly. Their short arms were of equal length, with no apparent extension of one arm beyond the other. This, together with the regular synaptic associations of these two chromosomes throughout their entire length, indicated that they were essentially homologous. If the above explanation is correct, until secondary exchanges occurred, 21-chromosome knob-bearing plants would be expected to possess the modified Zea and Tripsacum chromosomes and would therefore always show a high trivalent frequency. By this explanation it could be assumed that the plant described in previous studies in which the knob region was always paired in trivalent configurations, actually contained a normal Tripsacum chromosome and a modified Zea chromosome 2. This modified Zea chromosome would have carried the knob from the Tripsacum chromosome as a result of crossing over (between unmodified Zea and Tripsacum chromosomes) in an earlier generation and the distribution of crossover chromatids to the same pole. The situation is, of course, further complicated by the anomalous changes in knob position previously described, in which univalent Tripsacum chromosomes carried

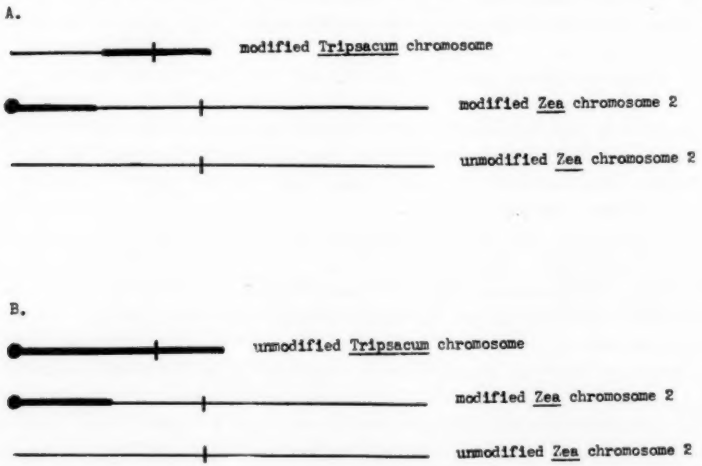

FIGURE 3.—Hypothetical types of chromosomes involved in trivalent configurations: A.—chromosomes of present study. B.—chromosomes of previous study. Chromosome parts originally from Tripsacum are indicated by a heavy line. Approximate centromere positions are shown by vertical crosslines.

the terminal knob in some sporocytes of a plant although in other sporocytes of the same plant this knob apparently was more often located on the Zea chromosome. In fact, the univalent condition of such Tripsacum chromosomes might be directly attributed to their possession of the knob and/or adjacent genetic material.

Genetic studies: Types of genetic segregation among 21-chromosome plants following trivalent formation and crossing over between Zea chromosome 2 and the Tripsacum chromosome in the *Lg—Gl* region are illustrated in figure 2. Two modes of synapsis and anaphase I distribution are illustrated. A study of the shape of metaphase I trivalents was inconclusive in determining the actual frequencies of these two types of anaphase I distribution, since many trivalents could not be classified as clearly indicating either type of distribution. A third type of anaphase I distribution in which the two Zea chromosomes disjoin from the Tripsacum chromosome could have been distinguished from the other two types in the study of metaphase I trivalent shapes, but was not found.

In order to compute expected genetic segregation and recombination ratios for the T_2 generation a number of tentative assumptions and approximations were made. It was assumed from a preliminary study of segregation ratios that the Tripsacum chromosome in stocks P5401 and P5403 carried dominant *Lg* and *Gl* alleles while the Tripsacum chromosome in stock P5402 did not carry these dominant genes. It was also assumed that the distribution of crossing over frequency is approximately uniform along the physical length of Zea chromosome 2. Thus since about 23 percent of the total length of Zea chromosome 2 (128 units) was usually paired with the Tripsacum chromosome in trivalent associations, this could have included the *Gl* locus and all loci distal to *Gl* but probably few or none proximal to it. Since there are 19 units between *Gl* and *Lg* and 30 between *Gl* and *Ws*, the most distal locus known in this arm of chromosome 2 (RHOADES 1955), it was assumed that about 63 percent of the total crossing over between the Tripsacum and Zea chromosomes occurred between *Gl* and *Lg*. In addition it was assumed that the metaphase I trivalent frequency found in microsporocytes of the female parent plants was a reliable index of the total crossing over frequency between the Tripsacum and Zea chromosomes in megasporocytes of these same plants, with no appreciable frequency of double crossing over. From a preliminary study of segregation ratios and from the pachytene observations described earlier it could be assumed that there was preferential pairing of the Tripsacum chromosome with the unmodified Zea chromosome from the male parent which carried recessive *lg* and *gl* alleles in stocks P5401 and P5403, so that pairing type I of figure 2 predominated. In P5402 it was similarly assumed that a secondary exchange had placed foreign Tripsacum genetic material on the Zea chromosome carrying the recessive *lg* and *gl* alleles so that pairing type II of figure 2 predominated. The observed segregation ratios suggested equal frequency of the two anaphase I types of distribution shown in figure 2. It was apparent from cytological observations of metaphase and early anaphase I studies that there was disjunction of the two Zea chromosomes 2 in all or nearly all sporocytes. The expected segregation ratios of table 3 were derived from the following formulae[3], where A is the trivalent frequency in the female parent of the stock (.90, .95 and .88 respectively

[3] Many of the fractional coefficients in these formulae can be most easily arrived at from a study of figure 2.

for stocks P5401, P5402 and P5403), B is the proportion of 21-chromosome progeny (table 1), C is the proportion of 20-chromosome progeny (table 1), and D is the univalent frequency in the female parent:

proportion $Lg = 7/8(.63)AB + 8/8(.37)AB + 6/8(.63)AC + 4/8(.37)AC + 8/8DB + 4/8DC$
in P5401 $= .79$
in P5403 $= .77$
proportion $Lg = 5/8(.63)AB + 4/8(.37)AB + 2/8(.63)AC + 4/8(.37)AC + 4/8DB + 4/8DC$
in P5402 $= .43$
proportion $Gl = 8/8AB + 4/8AC + 8/8DB + 4/8DC$
in P5401 $= .75$
in P5403 $= .72$
proportion $Gl = 4/8AB + 4/8AC + 4/8DB + 4/8DC$
in P5402 $= .50$
proportion $V = .50$
(in all stocks)

Chi square tests of the observed ratios (table 3) indicate that they are not inconsistent with the above assumptions at the five percent level.

Expected recombination ratios, listed in table 4, were derived from the following formulae where symbols A, B, C and D were used as above:
Expected recombination
in *Lg—Gl* region:

(in P5401 and P5403) $= 1/8(.63)AB + 2/8(.63)AC + .19DC$
$= .12$
(in P5402) $= 3/8(.63)AB + 2/8(.63)AC + .19D$
$= .19$

Chi square tests of observed recombination frequencies (table 4) indicate that none of them is inconsistent with the above assumptions at the five percent level.

Recombination in the *Gl—V* region (table 5) was found to differ greatly among

TABLE 3

Genetic ratios

Stock no.	*Lg:lg*			*Gl:gl*			*V:v*		
	Observed	Expected	χ^2	Observed	Expected	χ^2	Observed	Expected	χ^2
P5401	69:15	66:18	.64	62:26	66:22	.97	45:43	44:44	.046
P5402	33:55	38:50	1.16	44:45	44.5:44.5	.01	38:51	44.5:44.5	1.88
P5403	66:17	64:19	.27	61:27	63:25	.22	41:44	42.5:42.5	.106
Total P5401, P5402, P5403	168:87	168:87	0	167:98	173.5:91.5	.70	124:138	131:131	.74

TABLE 4

Genetic recombination in Lg-Gl *region*

Stock no.	Ratio: recombination types : parental types		
	Observed	Expected	χ^2
P5401	11:74	10:75	.11
P5402	13:75	17:71	1.17
P5403	12:71	10:73	.46
Total P5401, P5402, P5403	36:220	37:219	.032

TABLE 5

Genetic recombination in Gl-V *region*

Stock no.	Ratio: recombination types: parental types		
	Observed	Expected	χ^2
P5401	31:57	44:44	7.68
P5402	26:63	44.5:44.5	15.2
P5403	35:49	42:42	2.34
Total P5401, P5402, P5403	92:169	130.5:130.5	22.6

the three stocks and usually was significantly less than 50 percent. Double crossing over undoubtedly reduced the recombination observed here, and the differences among the three stocks could have resulted from a variable amount of pachytene pairing of the two Zea chromosomes in this region. Although no determination of the average amount of pairing was possible, marked differences among sporocytes were noted in this respect.

The results indicate that frequencies of genetic recombination in the *Lg—Gl* region were not inconsistent with the very high frequencies of metaphase I chiasmata described above, but exact comparisons cannot be made, since in each case a wide range of crossover frequencies would have given approximately the same observable genetic recombination frequency. There are in the literature many reports of departures from expected genetic crossover frequencies. For example, MORGAN (1950) found crossing over per unit length to be disproportionately high for the distal half of the short arm of chromosome 2 of Zea, and MCCLINTOCK (1943) noted a similar effect in another of the Zea chromosomes. BARBER (1941), CALLAN (1949) and OKSALA (1952) have suggested that interference reduction may result from interruption of pairing and change of pairing partner. Disturbances of normal crossover frequency have been noted in polyploids or polysomics by BRIDGES and ANDERSON (1925), CALLAN (1949), DARLINGTON (1934), DARLINGTON and MATHER (1932), OKSALA (1952), REDFIELD (1930, 1932), RHOADES (1936), SCHULTZ and REDFIELD (1951), and STONE and MATHER (1932).

TABLE 6

Phenotypic effects

Character	20-chrom. plants		21-chrom. plants	
	$\bar{X}$	s.d.	$\bar{X}$	s.d.
No. of days to silking†	75.17	4.45	77.34	3.49
Node number†	9.99	1.16	8.97	1.13
Earshoot number*	1.48	.61	1.31	.55
No. of tillers†	.32	.62	.84	.77
No. of tassel branches†	10.97	3.14	8.84	2.74
Leaf length (cm) (leaf above uppermost earshoot)†	60.94	7.83	53.00	7.69
Leaf width (cm) (leaf above uppermost earshoot)*	7.20	1.48	6.53	1.12
Leaf length/width	8.07	1.23	7.76	2.15
Vein no.	18.11	2.45	17.73	2.43
Vein no./cm leaf width	2.40	.32	2.56	.75
Plant height (cm)†	110.90	8.76	82.10	6.31

* Difference between means of 20- and 21-chromosome plants significant (five percent level, *t* test).

† Difference between means of 20- and 21-chromosome plants very significant (one percent level, *t* test).

Phenotypic effect studies: The Tripsacum chromosome had no very striking phenotypic effect other than pollen sterility on Zea plants carrying it. However, *t* tests of measurements of quantitative characters show significant differences between 20- and 21-chromosome plants at the five percent level as indicated in table 6. As compared to 20-chromosome plants, the 21-chromosome plants showed: greater number of days to silking, smaller node number, smaller earshoot number, greater number of tillers, smaller number of tassel branches, smaller leaf length, smaller leaf width, and smaller plant height. With the possible exception of increased number of days to silking and increased tiller number noted in 21-chromosome plants, the influence of the Tripsacum chromosome could be attributed to general reduction of vigor as readily as to any specific influence of Tripsacum genes. Differences with respect to ratio of leaf length/width, vein number and vein number per centimeter leaf width were not significant. Most 21-chromosome plants (78/82) were completely, or nearly completely pollen sterile, whereas most 20-chromosome plants (97/101) were completely pollen fertile. No ear sterility was found in any plant. The pollen studies suggest that a gene on the Tripsacum chromosome acts as a dominant for pollen sterility when present in the Zea nucleus, with some recombination in the region between it and the centromere. Since a single chromosome derived from the Tripsacum complement can confer essentially complete pollen sterility, the assumption of MANGELSDORF and REEVES (1939) that estimates of extra chromosome number among progenies of Zea-Tripsacum hybrids backcrossed to Zea can be based on degree of pollen sterility is of questionable value.

SUMMARY

1. Cytological and genetical studies were made of synapsis and crossing over in progenies of Zea-Tripsacum hybrids hyperploid for a chromosome derived from Tripsacum which originally carried a terminal knob.

2. The terminal knob of the Tripsacum chromosome appeared to be occasionally transferred between the end of the long arm of the Tripsacum chromosome and the end of the short arm of Zea chromosome 2, by some mechanism other than normal crossing over.

3. A high frequency of trivalent formation involving non random pairing of the Tripsacum chromosome and Zea chromosome 2 was observed in knob-bearing 21-chromosome plants. This is consistent with the interpretation that in an earlier generation an exchange of material occurred between Zea chromosome 2 and the Tripsacum chromosome involving segments which differed somewhat but were sufficiently homologous to allow apparently normal pairing between them.

4. The observed genetic ratios suggested that the Tripsacum chromosome normally carries *Lg* and *Gl* loci in its long arm.

5. Chiasma formation usually followed pairing of the Tripsacum chromosome with Zea chromosome 2, at least in knob-bearing plants.

6. The frequency of genetic recombination between *Lg* and *Gl* loci was not inconsistent with the unexpectedly high frequency of metaphase I chiasmata involving these chromosomes.

7. Plants carrying the Tripsacum chromosome differed significantly from those not carrying it with respect to number of days to silking, node number, earshoot number, number of tillers, number of tassel branches, leaf length, leaf width, plant height and pollen fertility.

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SOME BIOMETRICAL FORMULAE FOR THE ANALYSIS OF QUANTITATIVE INHERITANCE SYSTEMS INVOLVING TWO HAPLOID OR INBRED DIPLOID PARENTS[1]

D. S. ROBSON

Cornell University, Ithaca, New York

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THE general model underlying all methods of quantitative inheritance analysis asserts that in any genetic population consisting of a mixture of genotypes the population frequency distribution of phenotypes is a corresponding mixture of the individual phenotypic distributions associated with each of the genotypes. Thus, if G is a genetic population consisting of the n different genotypes $g_1, g_2, \cdots, g_n$ mixed with the relative frequencies $P(g_1 \mid G), P(g_2 \mid G), \cdots, P(g_n \mid G)$, then the relative frequency $f(x \mid G)$ of the phenotype x in the population G is

$$(1) \quad f(x \mid G) = P(g_1 \mid G) \cdot f(x \mid g_1) + P(g_2 \mid G) \cdot f(x \mid g_2) + \cdots + P(g_n \mid G) \cdot f(x \mid g_n).$$

By analogy to qualitative inheritance analysis, the complete solution of a quantitative inheritance problem includes the determination of (a) all the genotypes g occurring in the system, (b) the phenotypic characterization of each genotype g by means of its distribution function $f(x \mid g)$, and (c) the relative frequency $P(g \mid G)$ of the genotype g in each genetic population G of the system. In view of the many complicating factors known to occur in qualitative inheritance analysis this solution to a quantitative problem appears unobtainable; examples of quantitative heritable characters have been found, however, for which the genetic system was sufficiently simple to permit the fulfillment of (a), (b), and (c). Two such examples are given by ALLARD (1956) and POWERS (1955). Furthermore, where a complete solution is unobtainable a partial or approximate solution may be possible and, for some practical purposes, sufficient.

This paper is directed toward a partial solution of quantitative inheritance problems involving two parents, haploids or inbred diploids, and the population series generated from their F_1 cross by further crossing and selfing. The partial solution aimed for is the determination of the number N of loci at which the two parents are genetically different (with respect to the character in question) and the estimation of phenotypic frequency distributions associated with particular *collections* of genotypes, as opposed to *individual* genotypes. The formulas developed here may be regarded as generalizations of certain aspects of the *partitioning method of genetic analysis* described by POWERS (1934).

BACKCROSSING IN HAPLOIDS

Haploid organisms, particularly the lower forms, are convenient genetic material for the study of quantitative inheritance. There is, for example, the obvious advantage of the simplicity of haploid inheritance, the relatively short time interval

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between generations, the ability to produce large numbers of progeny, usually both sexually and asexually, and a life form which permits the experimental manipulation of the large numbers of individuals essential to a quantitative inheritance study. Backcrossing, starting from the cross of two parental genotypes, produces a series of generations in which the number of populations per generation increases geometrically, resulting in a rapid accumulation of the necessary data.

With respect to a single pair of alleles, say A_0 and A_1, the genotypic distribution in the two parental lines and their F_1 cross is

$$P(A_0 \mid P_0) = 1$$
$$P(A_1 \mid P_1) = 1$$
$$P(A_1 \mid F_1) = \tfrac{1}{2} = P(A_0 \mid F_1).$$

The two first generation backcross populations, $B_0 = F_1 \times P_0$ and $B_1 = F_1 \times P_1$, contain the genotypes A_0 and A_1 in the proportions

$$P(A_1 \mid B_0) = \tfrac{1}{4} = 1 - P(A_0 \mid B_0)$$
$$P(A_1 \mid B_1) = \tfrac{3}{4} = 1 - P(A_0 \mid B_1).$$

If we let $\delta_1 = 0$ or 1 according as the first backcross is to parent P_0 or P_1 then we may denote a general first backcross population by

$$B_{\delta_1} = F_1 \times P_{\delta_1}$$

and its genotypic frequency distribution by

$$P(A_1 \mid B_{\delta_1}) = \frac{1 + 2\delta_1}{2^2} = 1 - P(A_0 \mid B_{\delta_1}).$$

Extending this notation, we designate a general second generation backcross population by the symbol

$$B_{\delta_1,\delta_2} = B_{\delta_1} \times P_{\delta_2}$$

and it is easy to verify that the genotypic distribution in this population may be written

$$P(A_1 \mid B_{\delta_1,\delta_2}) = \frac{1 + 2\delta_1 + 2^2\delta_2}{2^2} = 1 - P(A_0 \mid B_{\delta_1,\delta_2}).$$

In general, then, we denote a kth generation backcross population by

$$B_{\delta_1,\cdots,\delta_k} = B_{\delta_1,\cdots,\delta_{k-1}} \times P_{\delta_k}$$

where $\delta_1, \cdots, \delta_k$ gives the complete ancestry of the population, and we have

$$P(A_1 \mid B_{\delta_1,\cdots,\delta_k}) = \frac{1 + 2\delta_1 + 2^2\delta_2 + \cdots + 2^k\delta_k}{2^{k+1}} = 1 - P(A_0 \mid B_{\delta_1,\cdots,\delta_k}).$$

With this understanding we shall simplify the notation by writing simply B for $B_{\delta_1,\cdots,\delta_k}$ and a for $P(A_1 \mid B_{\delta_1,\cdots,\delta_k})$.

N independently segregating loci. The two parental genotypes will, in general, differ at some unknown number N of loci, and we denote these two genotypes by

$$P_0^{(N)} = A_{01}A_{02} \cdots A_{0N}$$

$$P_1^{(N)} = A_{11}A_{12} \cdots A_{1N}.$$

The F_1 and every backcross population each contain all of the 2^N different possible genotypes, and if segregation is independent at the N loci then the relative frequency of any given genotype in the population B is obtained simply as the coefficient of that genotype in the expansion of

$$\prod_{j=1}^{N} [(1 - a)A_{0j} + aA_{1j}].$$

Upon expanding we find that every genotype carrying v genes from parent P_1 and $N - v$ genes from parent P_0 occurs with the same relative frequency, namely, $a^v(1 - a)^{N-v}$. The number of such genotypes is

$$\binom{N}{v} = \frac{N(N - 1) \cdots (N - v + 1)}{v(v - 1) \cdots 1};$$

hence, the probability that an individual randomly selected from the population B will carry v genes from parent P_1 and $N - v$ genes from parent P_0 is

$$\binom{N}{v}a^v(1 - a)^{N-v}.$$

We shall use the notation $P_1^{(v)}P_0^{(N-v)}$ to denote this genetic subpopulation consisting of $\binom{N}{v}$ equally frequent genotypes, so we may write

$$P(P_1^{(v)}P_0^{(N-v)} \mid B) = \binom{N}{v}a^v(1 - a)^{N-v}.$$

The fundamental assumption (1) then asserts that the phenotypic frequency distribution for the population B may be written as

$$f(x \mid B) = \sum_{v=0}^{N} \binom{N}{v}a^v(1 - a)^{N-v}f(x \mid P_1^{(v)}P_0^{(N-v)}). \tag{2}$$

Thus, the theoretical phenotypic frequency distribution for each backcross population is expressible as a linear combination of the $N + 1$ unknown distributions $f(x \mid P_1^{(v)}P_0^{(N-v)})$, $v = 0, 1, \cdots, N$.

The observed phenotypic frequency distribution for population B does not satisfy equation (2), but is subject to sampling error. If n is the number of individuals in the experimental population B and $f_n(x \mid B)$ is the fraction of these individuals in a given phenotypic class x the error

$$e(x) = f_n(x \mid B) - f(x \mid B)$$

is subject to a sampling variance of

$$\sigma_{e(x)}^2 = \frac{f(x \mid B)\,[1 - f(x \mid B)]}{n}.$$

This random error is added to both sides of (2) to give

$$(3)\quad f_n(x \mid B) = f(x \mid B) + e(x) = \sum_{v=0}^{N} \binom{N}{v} a^v (1-a)^{N-v} f(x \mid P_1^{(v)} P_0^{(N-v)}) + e(x).$$

A genetic experiment containing all of the possible backcross populations through generation k produces $2^{k+1} + 1$ different equations of the form (3), including those for the two parents and the F_1. When N is known and $N \leq 2^{k+1}$ the statistical solution to these equations represents a classical regression problem. The normalized equations obtained by dividing both sides of (3) by $\sigma_{e(x)}$,

$$(4)\quad \frac{f_n(x \mid B)}{\sigma_{e(x)}} = \sum_{v=0}^{N} \frac{\binom{N}{v} a^v (1-a)^{N-v}}{\sigma_{e(x)}} f(x \mid P_1^{(v)} P_0^{(N-v)}) + \frac{e(x)}{\sigma_{e(x)}},$$

satisfy the conditions of Markov's theorem on least squares, and the limiting distribution of the residual sum of squares,

$$\sum_{B} \left[\frac{f_n(x \mid B)}{\sigma_{e(x)}} - \sum_{v=0}^{N} \frac{\binom{N}{v} a^v (1-a)^{N-v}}{\sigma_{e(x)}} \hat{f}(x \mid P_1^{(v)} P_0^{(N-v)}) \right]^2,$$

where $\hat{f}(x \mid P_1^{(v)} P_0^{(N-v)})$ is the least squares solution to (4), is the chi square distribution with $2^{k+1} - N$ degrees of freedom. A comparison of the residual sum of squares with the tabulated chi square therefore constitutes a test of the model. Conventionally, when the weighting factors, in this case $\sigma_{e(x)}$, are unknown they are replaced by their estimates, here

$$(5)\quad \hat{\sigma}_{e(x)}^2 = \frac{f_n(x \mid B)\,[1 - f_n(x \mid B)]}{n}.$$

The model further implies that the parameters $f(x \mid P_1^{(v)} P_0^{(N-v)})$ being estimated here are non negative and that, when summed over all phenotypic classes x, each adds to unity. Since these conditions are not imposed upon the estimation procedure their fulfillment or nonfulfillment constitutes a further, informal test of the model.

This method of solution applies when N is known; but for a given choice of parental material, P_0 and P_1, there is exactly one value of N which gives a solution to (2) for all k. When N is unknown, therefore, the integer $\hat{N}$ which, by trial and error, yields a minimum residual sum of squares and non negative solutions $\hat{f}(x \mid P_1^{(v)} P_0^{(N-v)})$ is an estimate of N. Trial and error, however, where each trial consists of a multiple regression analysis, is a tedious process. To facilitate the estimation of N we may compute the algebraic solutions to sets of $N + 1$ equations of the form (2) and so establish linear relationships among the phenotypic frequency distributions $f(x \mid B)$ which, again, hold for exactly one value of N. The two recurrent backcross population series are of particular interest in this connection because of the convenient form of the algebraic solutions for $f(x \mid P_0^{(N)})$ and $f(x \mid P_1^{(N)})$.

The population $B_{\delta_1, \cdots, \delta_k}$ for which $\delta_1 = \delta_2 = \cdots = \delta_k = \delta$ is called the kth recurrent backcross population where P_δ is the recurrent parent, $\delta = 0$ or 1, and will be denoted by the symbol B_δ^k. The phenotypic frequency distribution for population B_δ^k has the simple form

$$(6)\quad f(x \mid B_\delta^k) = \sum_{v=0}^{N} \binom{N}{v} \left(\frac{1}{2^{k+1}}\right)^v \left(1 - \frac{1}{2^{k+1}}\right)^{N-v} f(x \mid P_{1-\delta}^{(v)}\, P_\delta^{(N-v)}).$$

The $N + 1$ equations for the successive generations B_δ^k, B_δ^{k+1}, $\cdots$, B_δ^{k+N} are easily solved to give, for all $k \geq 0$,

$$f(x \mid P_\delta^{(N)}) = \frac{\sum_{v=0}^{N} (-1)^v \alpha_v(N) f(x \mid B_\delta^{k+N-v})}{\sum_{v=0}^{N} (-1)^v \alpha_v(N)} \tag{7}$$

where the constants $\alpha_v(N)$, $v = 0, 1, \cdots, N$, are determined from the conditions

$$\alpha_0(N) = 1$$

$$\alpha_v(N) = \frac{1}{2^v}[\alpha_{v-1}(N-1) + \alpha_v(N-1)], \qquad 0 < v < N$$

$$\alpha_N(N) = \frac{1}{\frac{N(N+1)}{2}}$$

$$\alpha_{N+i}(N) = 0, \qquad i > 0.$$

For example,

$$\alpha_0(1) = 1, \quad \alpha_1(1) = \tfrac{1}{2}$$

$$\alpha_0(2) = 1, \quad \alpha_1(2) = \tfrac{3}{4}, \quad \alpha_2(2) = \tfrac{1}{8}$$

$$\alpha_0(3) = 1, \quad \alpha_1(3) = \tfrac{7}{8}, \quad \alpha_2(3) = \tfrac{7}{32}, \quad \alpha_3(3) = \tfrac{1}{64}.$$

As an alternative to equation (6) we have the recursion formula

$$f(x \mid B_\delta^{k+N+1}) = \sum_{v=0}^{N} (-1)^v 2^{v+1} \alpha_{v+1}(N+1) f(x \mid B_\delta^{k+N-v}); \tag{8}$$

thus, if the two parents are differentiated by $N = 1$ gene then for all $k \geq 0$

$$f(x \mid B_\delta^{k+2}) = \tfrac{3}{2} f(x \mid B_\delta^{k+1}) - \tfrac{1}{2} f(x \mid B_\delta^{k})$$

while for $N = 2$

$$f(x \mid B_\delta^{k+3}) = \tfrac{7}{4} f(x \mid B_\delta^{k+2}) - \tfrac{7}{8} f(x \mid B_\delta^{k+1}) + \tfrac{1}{8} f(x \mid B_\delta^{k}) \tag{9}$$

and for $N = 3$

$$f(x \mid B_\delta^{k+4}) = \tfrac{15}{8} f(x \mid B_\delta^{k+3}) - \tfrac{35}{32} f(x \mid B_\delta^{k+2}) + \tfrac{15}{64} f(x \mid B_\delta^{k+1}) - \tfrac{1}{64} f(x \mid B_\delta^{k}). \tag{10}$$

Formula (8), with f replaced by f_n, may then be used in place of (4) to obtain the estimate $\hat{N}$ by trial and error. The procedure would be to first see if the consecutive recurrent backcross populations fit the model with $N = 1$; if not, then try $N = 2$, and so on. Each relation (8) may be tested by comparing the statistic

$$t = \frac{f_n(x \mid B_\delta^{k+N+1}) - \sum_{v=0}^{N} (-1)^v 2^{v+1} \alpha_{v+1}(N+1) f_n(x \mid B_\delta^{k+N-v})}{\sqrt{\hat{\sigma}_{e(x)}^2(B_\delta^{k+N+1}) + \sum_{v=0}^{N} 4^{v+1} \alpha_{v+1}^2 (N+1) \hat{\sigma}_{e(x)}^2 (B_\delta^{k+N-v})}} \tag{11}$$

with the normal deviate $t_{.05} = 1.96$. The variance estimates $\hat{\sigma}_{e(x)}{}^2(B_\delta{}^m)$ are computed from formula (5); formula (11) indicates that each experimental population $B_\delta{}^m$ contains the same number n of individuals, but this is merely a notational convenience and is not necessary.

Any linear relationship which holds among frequency distributions likewise holds for the moments of the frequency distributions. The rth moment of a frequency distribution $f(x)$, written $\mu_r'(f)$, is defined as

$$\mu_r'(f) = \sum_x x^r f(x).$$

Consequently, equation (6) implies that

$$\mu_r'(B) = \sum_{v=0}^{N} \binom{N}{n} a^v (1 - a)^{N-v} \mu_r'(P_1{}^{(v)} P_0{}^{(N-v)}). \tag{12}$$

Since the rth sample moment for population B,

$$\hat{\mu}_r'(B) = \sum_x x^r f_n(x \mid B),$$

is an unbiased estimate of $\mu_r'(B)$ with variance

$$\sigma_{\hat{\mu}_r'}{}^2(B) = \frac{1}{n} \sum_x [x^r - \mu_r'(B)]^2 f(x \mid B)$$

then, writing $u(B) = \hat{\mu}_r'(B) - \mu_r'(B)$, the normalized equation corresponding to (4) becomes

$$\frac{\hat{\mu}_r'(B)}{\sigma_{\hat{\mu}_r'(B)}} = \sum_{v=0}^{N} \frac{\binom{N}{v} a^v (1 - a)^{N-v}}{\sigma_{\hat{\mu}_r'(B)}} \cdot \mu_r'(P_1{}^{(v)} P_0{}^{(N-v)}) + \frac{u(B)}{\sigma_{\hat{\mu}_r'(B)}}$$

which, again, may be attacked by the multiple regression analysis, substituting for $\sigma_{\hat{\mu}_r'(B)}$ the estimate obtained from

$$\hat{\sigma}_{\hat{\mu}_r'(B)}{}^2 = \frac{1}{n} \sum_x [x^r - \hat{\mu}_r'(B)]^2 f_n(x \mid B).$$

Similarly, the relations (7) and (8) hold for the moments of the recurrent backcross populations; corresponding to (8) we have the recursion

$$\mu_r'(B_\delta{}^{k+N+1}) = \sum_{v=0}^{N} (-1)^v 2^{v+1} \alpha_{v+1} (N + 1) \mu_r'(B_\delta{}^{k+N-v})$$

which may be tested by the criterion

$$t = \frac{\hat{\mu}_r'(B_\delta{}^{k+N+1}) - \sum_{v=0}^{N} (-1)^v 2^{v+1} \alpha_{v+1} (N + 1) \hat{\mu}_r'(B_\delta{}^{k+N-v})}{\sqrt{\hat{\sigma}_{\hat{\mu}_r'}{}^2{}_{(B_\delta{}^{k+N+1})} + \sum_{v=0}^{N} 4^{v+1} \alpha_{v+1}{}^2 (N + 1) \hat{\sigma}_{\hat{\mu}_r'}{}^2{}_{(B_\delta{}^{k+N-v})}}}.$$

Thus, one method of determining the estimate $\hat{N}$ is by trial and error using the first one or two sample moments. For example, if the sample means $\bar{B}_\delta{}^k = \hat{\mu}_r'(B_\delta{}^k)$ satisfy the relations

$$\bar{B}_0{}^2 = \tfrac{3}{2}\,\bar{B}_0{}^1 - \tfrac{1}{2}\,\bar{F}_1 \qquad \bar{B}_1{}^2 = \tfrac{3}{2}\,\bar{B}_1{}^1 - \tfrac{1}{2}\,\bar{F}_1$$

$$\bar{B}_0{}^3 = \tfrac{3}{2}\,\bar{B}_0{}^2 - \tfrac{1}{2}\,\bar{B}_0{}^1 \qquad \bar{B}_1{}^3 = \tfrac{3}{2}\,\bar{B}_1{}^2 - \tfrac{1}{2}\,\bar{B}_1{}^1$$

$$\vdots \qquad\qquad\qquad \vdots$$

$$\bar{B}_0{}^{k+2} = \tfrac{3}{2}\,\bar{B}_0{}^{k+1} - \tfrac{1}{2}\,\bar{B}_0{}^k \qquad \bar{B}_1{}^{k+2} = \tfrac{3}{2}\,\bar{B}_1{}^{k+1} - \tfrac{1}{2}\,\bar{B}_1{}^k$$

to within sampling error this would strongly suggest that $N = 1$ would give the optimum solution to (4). Or if one or more of these relations does not hold but

$$\bar{B}_0{}^3 = \tfrac{7}{4}\,\bar{B}_0{}^2 - \tfrac{7}{8}\,\bar{B}_0{}^1 + \tfrac{1}{8}\,\bar{F}_1 \qquad \bar{B}_1{}^3 = \tfrac{7}{4}\,\bar{B}_1{}^2 - \tfrac{7}{8}\,\bar{B}_1{}^1 + \tfrac{1}{8}\,\bar{F}_1$$

$$\bar{B}_0{}^4 = \tfrac{7}{4}\,\bar{B}_0{}^3 - \tfrac{7}{8}\,\bar{B}_0{}^2 + \tfrac{1}{8}\,\bar{B}_0{}^1 \qquad \bar{B}_1{}^4 = \tfrac{7}{4}\,\bar{B}_1{}^3 - \tfrac{7}{8}\,\bar{B}_1{}^2 + \tfrac{1}{8}\,\bar{B}_1{}^1$$

$$\vdots \qquad\qquad\qquad \vdots$$

$$\bar{B}_0{}^{k+3} = \tfrac{7}{4}\,\bar{B}_0{}^{k+2} - \tfrac{7}{8}\,\bar{B}_0{}^{k+1} + \tfrac{1}{8}\,\bar{B}_0{}^k \qquad \bar{B}_1{}^{k+3} = \tfrac{7}{4}\,\bar{B}_1{}^{k+2} - \tfrac{7}{8}\,\bar{B}_1{}^{k+1} + \tfrac{1}{8}\,\bar{B}_1{}^k$$

were all satisfied to within sampling error then the conclusion would be that $N = 2$ should give the best solution to (4), and so on.

Two linked genes. If the parents P_0 and P_1 differ in exactly two genes controlling the quantitative character, both of which occur on the same chromosome, this fact is relatively easy to demonstrate by recurrent backcrossing. Letting $p/2$ denote the relative frequency of each parental genotype in the F_1 population we may write the phenotypic frequency distribution for the kth recurrent backcross population $B_\delta{}^k$ as

$$f(x \mid B_\delta{}^k) = \left[1 - \frac{1}{2^k} + \left(\frac{p}{2}\right)^{k+1}\right] f(x \mid P_\delta{}^{(2)}) + \frac{1 - p^{k+1}}{2^{k+1}}\, f(x \mid P_1{}^{(1)}\, P_0{}^{(1)}) + \left(\frac{p}{2}\right)^{k+1} f(x \mid P_{1-\delta}{}^{(2)}).$$

The equations for the three populations $B_\delta{}^k$, $B_\delta{}^{k+1}$, $B_\delta{}^{k+2}$ then give the solution

$$f(x \mid P_\delta{}^{(2)}) = \frac{f(x \mid B_\delta{}^{k+2}) - \dfrac{1 + p}{2}\, f(x \mid B_\delta{}^{k+1}) + \dfrac{p}{4}\, f(x \mid B_\delta{}^k)}{1 - \dfrac{1 + p}{2} + \dfrac{p}{4}} \tag{13}$$

Alternatively, since $f(x \mid P_\delta{}^{(2)}) = f(x \mid P_\delta)$ can be estimated from the experimental population P_δ, we may regard (13) as a solution for p; namely,

$$\frac{p}{2} = \frac{2f(x \mid B_\delta{}^{k+2}) - f(x \mid B_\delta{}^{k+1}) - f(x \mid P_\delta)}{2f(x \mid B_\delta{}^{k+1}) - f(x \mid B_\delta{}^k) - f(x \mid P_\delta)} \tag{14}$$

for all k. The recursion corresponding to (8) is then

$$f(x \mid B_\delta{}^{k+3}) = \frac{p + 3}{2}\, f(x \mid B_\delta{}^{k+2}) - \frac{2 + 3p}{4}\, f(x \mid B_\delta{}^{k+1}) + \frac{p}{4}\, f(x \mid B_\delta{}^k). \tag{15}$$

Formulas (14) and (15) likewise hold with $f(x \mid B_\delta^m)$ replaced by the rth moment $\mu_r'(B_\delta^m)$.

If linkage occurs when $N > 2$ the problem becomes much more complicated. For example, if $N = 3$ genes are distributed over two chromosomes then the relations corresponding to (13) and (15) become, respectively,

$$f(x \mid P_\delta^{(3)}) = \frac{1}{24 - 18p + 3p^2}\,[64f(x \mid B_\delta^{k+4}) - 48(1 + p)f(x \mid B_\delta^{k+3}) + (8 + 36p + 8p^2)f(x \mid B_\delta^{k+2}) - 6p(1 + p)f(x \mid B_\delta^{k+1}) + p^2 f(x \mid B_\delta^{k})] \tag{16}$$

$$f(x \mid B_\delta^{k+5}) = \frac{7 + 3p}{4} f(x \mid B_\delta^{k+4}) - \frac{14 + 21p + 2p^2}{16} f(x \mid B_\delta^{k+3}) + \frac{4 + 21p + 7p^2}{32} f(x \mid B_\delta^{k+2}) - \frac{p(6 + 7p)}{64} f(x \mid B_\delta^{k+1}) + \frac{p^2}{64} f(x \mid B_\delta^{k}) \tag{17}$$

where p has the same meaning as before with respect to the two linked genes. Equation (16) is quadratic in p and, in general, will be of degree $N - 1$ in p if the N genes occur on $N - 1$ chromosomes. Also, we observe that the recursion (17) involves the frequency distributions for six different populations as compared to five in the corresponding expression (10) for the case of $N = 3$ independently segregating genes; this is due to the fact that all genotypes carrying v genes from parent P_1 and $N - v$ from parent P_0 no longer appear with equal frequency in the backcross populations.

BACKCROSSING AND SELFING IN DIPLOIDS

The formulas presented above for a haploid backcrossing system also apply, with appropriate changes in notation, to a diploid system of backcrossing and selfing starting with the cross of two homozygous parents. If the N factors by which the two parents differ (with respect to the quantitative character in question) segregate independently then in the population $B_{\delta_1,\cdots,\delta_k,0}$ the $\binom{N}{v}$ different genotypes which are heterozygous at exactly v loci and hence homozygous for P_0 genes at the remaining $N - v$ loci all appear with equal frequency; namely, $a^v(1 - a)^{N-v}$ where, again,

$$a = \frac{1 + 2\delta_1 + 2^2\delta_2 + \cdots + 2^k\delta_k}{2^{k+1}}.$$

Hence, if we define $F_1^{(v)}P_0^{(N-v)}$ to be the subpopulation consisting of a uniform mixture of these $\binom{N}{v}$ different genotypes then the preceding formulas for haploids apply with $f(x \mid P_1^{(v)}P_0^{(N-v)})$ replaced by $f(x \mid F_1^{(v)}P_0^{(N-v)})$. Likewise, replacing $f(x \mid P_1^{(v)}P_0^{(N-v)})$ by $f(x \mid P_1^{(v)}F_1^{(N-v)})$ gives the formulas for the diploid backcross population $B_{\delta_1,\cdots,\delta_k,1}$. If the population $B_{\delta_1,\cdots,\delta_k,0}$ is selfed for r consecutive generations following the $(k + 1)$th backcross then in the resulting population, denoted by $B_{\delta_1,\cdots,\delta_k,0\otimes r}$, the relative frequency of any particular genotype is given by the coefficient of that genotype in the expansion of

$$\prod_{j=1}^{N}\left\{\left[(1 - a) + \frac{a}{2}\left(1 - \frac{1}{2^r}\right)\right]A_{0j}A_{0j} + \frac{a}{2^r}A_{0j}A_{1j} + \frac{a}{2}\left(1 - \frac{1}{2^r}\right)A_{1j}A_{1j}\right\}.$$

Clearly, every genotype which is heterozygous at exactly v loci and homozygous for P_0 genes at exactly β loci (and hence homozygous for P_1 genes at exactly $N - v - \beta$ loci) appears with the same relative frequency; namely,

$$\left[\frac{a}{2^r}\right]^v \left[(1-a) + \frac{a}{2}\left(1 - \frac{1}{2^r}\right)\right]^\beta \left[\frac{a}{2}\left(1 - \frac{1}{2^r}\right)\right]^{N-v-\beta}.$$

The number of such genotypes is

$$\frac{N!}{v!\beta!(N-v-\beta)!}$$

so, letting $F_1^{(v)}P_0^{(\beta)}P_1^{(N-v-\beta)}$ denote the subpopulation consisting of these genotypes mixed with equal frequency, we may write

$$P(F_1^{(v)}P_0^{(\beta)}P_1^{(N-v-\beta)}) = \frac{N!}{v!\beta!(N-v-\beta)!}\left[\frac{a}{2^r}\right]^v \left[(1-a) + \frac{a}{2}\left(1 - \frac{1}{2^r}\right)\right]^\beta \left[\frac{a}{2}\left(1 - \frac{1}{2^r}\right)\right]^{N-v-\beta}.$$

The fundamental assumption (1) then gives the phenotypic distribution in population $B_{\delta_1,\cdots,\delta_k,0\otimes r}$ as

$$f(x \mid B_{\delta_1,\cdots,\delta_k,0\otimes r}) = \sum_{v=0}^{N}\sum_{\beta=0}^{N-v} P(F_1^{(v)}P_0^{(\beta)}P_1^{(N-v-\beta)}) f(x \mid F_1^{(v)}P_0^{(\beta)}P_1^{(N-v-\beta)}).$$

Thus, the theoretical phenotypic frequency distribution is expressed as a linear function of the $(N+1)(N+2)/2$ unknown distributions $f(x \mid F_1^{(v)}P_0^{(\beta)}P_1^{(N-v-\beta)})$. Regression estimation of these unknowns requires the inclusion of at least $(N+1)(N+2)/2$ populations of the form $B_{\delta_1,\cdots,\delta_k,\delta_{k+1}\otimes r}$ in the genetic experiment, where N, again, may be estimated from the recursion (8) which holds also for diploids.

The selfing series offers the same advantages as the recurrent backcrosses in that the phenotypic frequency distribution of population $F_{k+1} = F_{k\otimes}$ is expressible as a linear function of $N+1$ unknown distributions, and the series $F_1, F_2, F_3, \cdots$ satisfies the same recursion (8) as the recurrent backcross series. In F_{k+1} the relative frequency of each of the $\binom{N}{v}2^{N-v}$ genotypes which are heterozygous at exactly v loci is

$$\left(\frac{1}{2^k}\right)^v \left[\frac{1}{2}\left(1 - \frac{1}{2^k}\right)\right]^{N-v}.$$

Letting $F_1^{(v)}$ denote the uniform mixture of these genotypes we may then write the phenotypic distribution for F_{k+1} as

$$f(x \mid F_{k+1}) = \sum_{v=0}^{N} \binom{N}{v}\left(\frac{1}{2^k}\right)^v \left(1 - \frac{1}{2^k}\right)^{N-v} f(x \mid F_1^{(v)}).$$

The solution corresponding to (7) is then

$$f(x \mid F_1^{(0)}) = \frac{\sum_{v=0}^{N}(-1)^v \alpha_v(N) f(x \mid F_{k+N-v})}{\sum_{v=0}^{N}(-1)^v \alpha_v(N)}$$

and the recursion corresponding to (8) is

$$f(x \mid F_{k+N+1}) = \sum_{v=0}^{N} (-1)^v 2^{v+1} \alpha_{v+1} (N + 1) f(x \mid F_{k+N-v}).$$

Linkage offers the same difficulties in diploid systems as in haploid systems; in fact, the recursions (15) and (17) derived for haploid recurrent backcrosses apply directly to diploid recurrent backcrosses. No simple recursion corresponding to (15) exists for the selfing series.

DISCUSSION

The fundamental relation (1) applies regardless of whether the phenotypes x are real numbers, ordered classes, or simply qualitative (unordered) classes; the existence of a natural ordering, however, sometimes permits the further partition of a distribution such as $f(x \mid F_1^{(v)} P_0^{(N-v)})$ into its component parts. It is fairly common to find that phenotypes are graded on a scale, say, from one to ten, and in this case we say a natural ordering of phenotypes exists but not a natural metric. When the estimated distribution $\hat{f}(x \mid F_1^{(v)} P_0^{(N-v)})$ has more than one mode with respect to the natural ordering of x then it is sometimes possible to visually pick out individual components of the distribution. Thus, in a diploid system involving two independently segregating loci the distribution $f(x \mid F_1^{(1)} P_0^{(1)})$ is a uniform mixture of two component distributions,

$$f(x \mid F_1^{(1)} P_0^{(1)}) = \tfrac{1}{2} f(x \mid A_0 A_0 B_0 B_1) + \tfrac{1}{2} f(x \mid A_0 A_1 B_0 B_0),$$

where $A_0 A_0 B_0 B_0$ is the genotype of parent P_0. If these two component distributions do not overlap with respect to the ordering of x then by plotting $\hat{f}(x \mid F_1^{(1)} P_0^{(1)})$ it may be possible to separate them. For example, if phenotypes are graded one to ten and the estimated distribution $\hat{f}(x \mid F_1^{(1)} P_0^{(1)})$ were

$x =$	1	2	3	4	5	6	7	8	9	10
$\hat{f}(x \mid F_1^{(1)} P_0^{(1)}) =$	.20	.30	.05	0	0	0	.05	.15	.20	.10

one would tentatively conclude that the two probability masses separated by an interval of no probability correspond to the two component distributions. Verification of this fact and identification of genotypes with component distributions could then be made by progeny tests; the statistical problems of progeny testing in a quantitative inheritance study, however, are not within the province of this paper. In its present stage of development, the *partitioning method* is dependent upon such visual decompositions for a complete solution to the inheritance problem; further statistical refinement of the method awaits the development of non parametric tests for linear combinations of distribution functions.

The successful application of the partitioning method and the general formulas given above requires data from a large number of individuals per population. POWERS (1955) has empirically arrived at the minimum figure of roughly 400 entries per segregating population. Another essential feature of the genetic experiment, as stressed by POWERS, is that all data be collected at one time, from one "location", and preferably from a completely randomized experimental design. This, of course, does not exclude

sequential experimentation in which at each stage of the sequence all previously derived populations are represented in the experiment. Complete randomization in the experimental design is the one basic assumption underlying the fundamental relation (1) and any restrictions upon the randomization such as the use of randomized blocks or even the use of "plots" of more than one individual per plot constitute violations of the model. Where these requirements lead to an experiment of unmanageable proportions because of an apparent large number of segregating genes the problem may be modified and simplified by selection to fix the genotype at some of the loci and so reduce the number N.

SUMMARY

Formulas are given which show some of the theoretic relationships among the phenotypic frequency distributions in a backcrossing and selfing system involving two haploid or homozygous diploid parents. The parents, say P_1 and P_0, are assumed to differ genotypically at some unknown number N of loci, and one of the primary uses of the formulas is to determine N. Linkage is excluded with the exception of systems involving exactly one pair of linked genes, and other complicating factors such as sexual dimorphism and differential viability of gametes or zygotes are assumed nonexistent.

A device used in obtaining the formulas is the partitioning of the collection of all possible genotypes into subsets such that in any population derived by the breeding system every genotype of a given subset appears with the same theoretic relative frequency. In the case of haploids any backcross population is a mixture of the 2^N possible genotypes, but when segregation is independent at the N loci every genotype which carries exactly v genes of parent P_1 and $N - v$ of parent P_0 appears with the same theoretic relative frequency. We call this collection of $\binom{N}{v}$ equally frequent genotypes the subpopulation $P_1^{(v)}P_0^{(N-v)}$ and regard each backcross population as a mixture of these $N + 1$ subpopulations. The relative frequency of the subpopulation $P_1^{(v)}P_0^{(N-v)}$ in the backcross population B obtained from the series of crosses $F_1 \times P_{\delta_1} \times P_{\delta_2} \times \cdots \times P_{\delta_k}$, where $\delta_i = 1$ or 0, is then $\binom{N}{v}a^v(1 - a)^{N-v}$ where

$$a = \frac{1 + 2\delta_1 + 2^2\delta_2 + \cdots + 2^k\delta_k}{2^{k+1}}.$$

Theoretically, then, the relative frequency $f(x \mid B)$ of the phenotype x in the population B is

$$f(x \mid B) = \sum_{v=0}^{N} \binom{N}{v} a^v(1 - a)^{N-v} f(x \mid P_1^{(v)}P_0^{(N-v)})$$

where the component $f(x \mid P_1^{(v)}P_0^{(N-v)})$ is the relative frequency of phenotype x in the subpopulation $P_1^{(v)}P_0^{(N-v)}$. Statistical techniques are suggested for estimating these component distributions and their moments, thus generalizing certain aspects of the *partitioning method of genetic analysis* described by POWERS.

The recurrent backcross population where $\delta_1 = \delta_2 = \cdots = \delta_k = \delta = 1$ or 0 is denoted by B_δ^k and this series is shown to satisfy

$$f(x \mid B_\delta^{k+N+1}) = \sum_{v=0}^{N} (-1)^v 2^{v+1} \alpha_{v+1}(N + 1) f(x \mid B_\delta^{k+N-v})$$

where $\alpha_{v+1}(N+1)$, $v = 0, 1, \cdots, N$, are known constants. For a given choice of parental material there is exactly one value of N such that this recursive relation holds for all k; hence, by testing the various possible recursions on the experimental data N may be estimated. For the case of $N = 2$ linked genes this recursion becomes

$$f(x \mid B_\delta^{k+3}) = \frac{p+3}{2} f(x \mid B_\delta^{k+2}) - \frac{2+3p}{4} f(x \mid B_\delta^{k+1}) + \frac{p}{4} f(x \mid B_\delta^{k})$$

where p is the unknown linkage parameter. With some slight changes in notation these same formulas are made to apply to the diploid system.

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THE GENETICS OF FLOWERING RESPONSE IN COTTON. I. FRUITING BEHAVIOR OF GOSSYPIUM HIRSUTUM VAR. MARIE-GALANTE IN A CROSS WITH A VARIETY OF CULTIVATED AMERICAN UPLAND COTTON[1]

C. F. LEWIS AND T. R. RICHMOND

Department of Agronomy, Texas Agricultural Experiment Station, College Station, Texas

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THE group of cottons, characterized as American Upland, *Gossypium hirsutum*, L., comprises a number of taxonomic varieties and geographic races. Southern Mexico and the northern part of Central America generally are recognized as the center of variability of American Upland cotton. It is from this area that the cultivated varieties of the Cotton Belt of the United States originated. Most of these varieties trace back to the colonial era but one important one, Acala, was extracted from a collection made in Mexico by COLLINS and DOYLE in 1907.

Within the past ten years there has been a renewed interest in the introduction of American Upland cottons from the center of variability. This interest stems primarily from a desire on the part of cotton biologists to obtain new and more variable basic breeding stocks. But another consideration is the realization that new and improved roads in the area are bringing about changes in the local economy which tend to supplant home spinning and weaving with imported textile products. The inevitable consequence, particularly in those areas where there is considerable pressure on land utilization for the production of food crops, has been a reduction in door-yard and small-field plantings of cotton. The possibility that many of the indigenous stocks would eventually become lost or extinct has further encouraged the collection and preservation of these materials.

Three collecting trips have been made into southern Mexico and Central America since 1946 and as a result some 600 native or primitive stocks of *G. hirsutum* have been introduced into the United States. More than half of these stocks failed to set fruit when grown during the summer at College Station, Texas. Representative samples of these same stocks flowered and fruited readily when grown in a greenhouse during the winter or when grown in frost-free locations in Cuba and Puerto Rico. Seeds of many of the introduced cottons were shared with J. B. HUTCHINSON of the Empire Cotton Growing Corporation, a leading authority on the taxonomy of cotton. According to HUTCHINSON (1951) there was no case of failure to flower in equatorial Uganda although differences in time of flowering were observed.

Since earlier cotton breeding work in the United States was confined to material grown in the field during the long days of summer, it is obvious that all nonflowering plants in introduced stocks were lost the very first year they were planted. It is well-known that day-length sensitive plants of this type can be induced to flower

[1] Contribution from Field Crops Research Branch, Agricultural Research Service, U. S. Department of Agriculture and Department of Agronomy, Texas Agricultural Experiment Station cooperating under Regional Cotton Genetics Project S-1.

by favorable environmental conditions. However, little is known of the extent to which flowering and fruiting response is under genetic control, the genetic mechanisms involved, or the linkage or association of these responses with certain other characters of economic value.

This paper is the first of a series in which the fruiting response of certain taxonomic varieties and geographic races of American Upland cotton will be reported. The experiment reported here was designed to study the genetics of fruiting response in a stock of *G. hirsutum* var. *marie-galante* Murry native to Central America.

REVIEW OF LITERATURE

The influence of environmental factors on fruiting in cotton has been reported by several investigators. According to HUTCHINSON, SILOW and STEPHENS (1947), *G. hirsutum* evolved in nature by spreading into three geographically and ecologically distinct areas, and three morphologically distinct varieties resulted. These varieties are typical *G. hirsutum*, commonly called American Upland cotton, and two taxonomic varieties, var. *punctatum* and var. *marie-galante*. After studying the recent collections of *G. hirsutum* from Central America, HUTCHINSON (1951) describes seven geographic races of *hirsutum*, but Upland, *punctatum* and *marie-galante* are still recognized as being distinct types of cotton. HUTCHINSON (1947) stated that these three varieties could be distinguished primarily on their fruiting habit in their natural habitat in the tropics and subtropics.

HUTCHINSON (1951), MAUER (1930), and WARE (1936) recognized that many of the Central American cottons in addition to *marie-galante*, were sufficiently sensitive to length of day to prevent them from fruiting satisfactorily when moved from the tropics to latitudes as far north as the Cotton Belt of the United States. The establishment of the Upland crop in the United States involved the selection of day-neutral stocks which would flower early in the long days of summer.

LANG (unpublished) found that many of the types of cotton which fail to flower in the summer at College Station, Texas could be made to flower by subjecting them to short photoperiods of nine or ten hours, and he considered them short-day plants. Differences in earliness remained, however, after photoperiod was no longer a limiting factor. LANG was also able to induce flowering in short-day types by grafting them on to a two branched, day-neutral donor and removing flower buds from the donor as soon as they developed.

Since GARNER and ALLARD (1920) reported the importance of length of day in determining the time of flowering in plants, many investigations have been made on the role of light in influencing initiation and development of flowers in plants. MURNEEK *et al.* (1948) wrote an extensive review and LEOPOLD (1951) prepared a condensed summary of work on photoperiodism and the interrelationship of other environmental factors on flowering in plants.

LANG and MELCHERS (1943) showed how the light requirement for flowering in photoperiod sensitive henbane is modified by changes in temperature. ROBERTS and STRUCKMEYER (1938) discussed the effect of temperature upon photoperiodic response in plants.

Genetic studies involving photoperiodic stocks have been conducted by several

investigators. ALLARD (1919) observed that gigantism or the mammoth character in *Nicotiana tabacum* was inherited as a simple recessive, although it was not until a year later that GARNER and ALLARD (1920) discovered that photoperiod was the determining environmental factor in the initiation of flowers in this material. LANG (1948) found that the short-day response of Maryland Mammoth was monofactorial and recessive.

QUINBY and KARPER (1945) reported that three genes conditioned response to photoperiod in milo and that four maturity types could be recognized under natural day-length conditions. SMITH (1950) reported that a recessive gene for short-day photoperiod response in *Nicotiana tabacum* gave a different response when transferred to *Nicotiana rustica* by backcrossing. ROGERS (1950) studied the inheritance of photoperiodic response in maize-teosinte hybrids involving a day-neutral maize parent and several short-day varieties of teosinte. Several genes were responsible for the photoperiodic response of each of the teosinte varieties. Backcrosses to the Guatemalan teosinte varieties approaches the teosinte parent in each instance while the maize backcross progenies of these same varieties approached the maize parent in time of flowering.

MATERIALS AND METHODS

The photoperiod sensitive stock used in this study was *G. hirsutum* var. *marie-galante* collected by S. G. STEPHENS in Santa Ana, Salvador. In his work with this strain LANG (unpublished) found that it flowered readily in a 10-hour photoperiod but remained strictly vegetative in natural days of summer at College Station. This *marie-galante* strain initiated the first visible fruit form at the fifteenth node, or higher, about ten weeks after planting as compared to the fourth to sixth node and fifth week in day-neutral Upland varieties. Thus, photoperiodic response and earliness were recognized as separate phenomena. The exact pedigree of this strain is not known but it was self-fertilized at least three generations before the authors obtained the seed. No segregation for photoperiodic response or any other character was observed.

An inbred line derived from a cultivated Upland variety, Deltapine-14, was used as the day-neutral parent. Deltapine-14 flowered readily either in the long days of summer or the short days of winter.

By utilizing a greenhouse, where the two parental strains could be induced to flower at the same time, appropriate crosses and self-pollinations were made to produce a supply of the following seed stocks:

1. *G. hirsutum* var. *marie-galante*
2. *G. hirsutum*–Deltapine-14
3. F_1 *marie-galante* × Deltapine-14
4. F_2 *marie-galante* × Deltapine-14
5. First backcross, F_1 to *marie-galante*
6. First backcross, F_1 to Deltapine-14

Plantings were made in such a way that behavior of each of the six populations, when grown during the long days of summer in the field, could be compared with similar populations grown during the short days of winter in the greenhouse. The

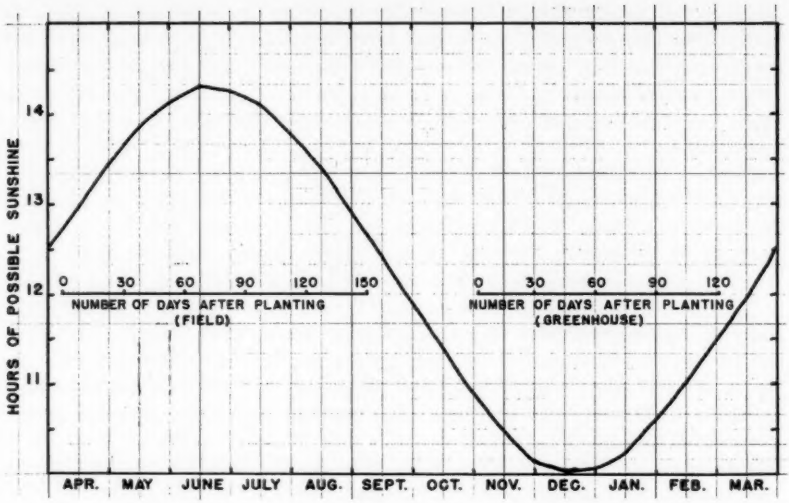
HOURS OF POSSIBLE SUNSHINE
14
13
12
11
0 30 60 90 120 150
NUMBER OF DAYS AFTER PLANTING (FIELD)
0 30 60 90 120
NUMBER OF DAYS AFTER PLANTING (GREENHOUSE)
APR. MAY JUNE JULY AUG. SEPT. OCT. NOV. DEC. JAN. FEB. MAR.

FIGURE 1.—The number of hours between sunrise and sunset during the year at College Station, Texas.

natural lengths of day in Texas during the course of the experiments are shown in figure 1. These two environmental conditions made it possible to take advantage of the natural, annual variation in length of day and to represent the actual situations in which work with the introduced cottons must be done. Facilities were not available for critical control of environmental factors; the data on the behavior of the plants actually represent their reaction to the total environment.

The field planting was designed as a randomized block with four replications, with 27 single row plots per replication. Each replication consisted of one plot each of the two parents and the F_1, 15 plots of F_2, three plots of the first backcross to *marie-galante* and six plots of the first backcross to Deltapine-14. Each plot contained ten plants spaced three feet apart making a possible total of 40 plants of each parent and the F_1, 600 of the F_2, 120 of the first backcross to *marie-galante* and 240 of the first backcrosses to Deltapine-14. Seeds for this planting were sown in 3-inch paper cups in the greenhouse on April 7, 1950 and seedlings were transplanted to the field at three weeks of age.

A randomized block design with three replications and 14 rows per replication was used for the greenhouse planting. A replication included one row of each of the two parents and the F_1, five rows of the F_2, three first backcrosses to *marie-galante* and three of the first backcross to Deltapine-14. Each row consisted of three 10-inch pots, making a total of nine plants of each of the parents and the F_1, 45 of the F_2, 27 first backcrosses to *marie-galante*, and 27 of the first backcrosses to Deltapine-14. Seeds for this study were sown in 3-inch paper cups on November 2, 1950 and seedlings were transplanted to 10-inch pots at two weeks of age.

TABLE 1

Summary of data on squaring and flowering behavior of six progenies of cotton grown during long days of summer in the field and during short days of winter in the greenhouse

Progeny	No. of plants under observation		Percent of field plants with		Days to first square[1]		Days to first flower[1]	
	Field	G.H.	Squares	Flowers	Field	G.H.	Field	G.H.
G. hirsutum var. *marie-galante*	22	9	0	0	No sq.	89 ± 3.1	No flower	131 ± 3.2
G. hirsutum, Deltapine-14	37	8	100	100	57 ± .2	48 ± .6	78 ± .5	77 ± 1.1
F_1 *marie-galante* × Deltapine-14	39	9	100	49	62 ± .6	52 ± 1.0	102 ± 2.0	85 ± 1.5
F_2 *marie-galante* × Deltapine-14	567	45	88	53	72 ± .9	60 ± 1.2	94 ± .7	94 ± 1.1
First backcross, F_1 to *marie-galante*	99	27	35	0	102 ± 4.8	75 ± 1.5	No flower	110 ± 2.0
First backcross, F_1 to Deltapine-14	230	27	100	100	58 ± .2	49 ± .6	81 ± .4	81 ± .7

[1] Mean of those plants which squared and flowered.

The flowering behavior of these plants was measured in two ways: (1) Recording the number of days elapsing between planting and the first observation of a visible fruit or "square". By careful examination, a young fruit form can be seen when it is 1–2 mm in length. (2) Recording the number of days elapsing between planting and anthesis of the first bloom.[2] The plants were examined twice a week in the early stages of growth, every second day at the peak of the flowering period, and about once a week later in the season.

No meiotic studies or reciprocal crosses were made. HUTCHINSON, SILOW and STEPHENS (1947) grew extensive F_2 cultures of *G. hirsutum* × *marie-galante* at Trinidad, and no unbalanced, sterile or morphologically abnormal segregates occurred. Meiotic studies of intra*hirsutum* crosses have all been essentially normal.

RESULTS

The two parental stocks, *marie-galante* and Deltapine-14, were strikingly different in their flowering behavior when grown in the field during the long days of summer; they also differed during the short days of winter. Table 1 shows that the *marie-galante* plants in the field produced no squares and, of course, no flowers while Deltapine-14 squared and flowered profusely. Photographs 1 and 2 in figure 2 show field grown plants of *marie-galante* and Deltapine-14 respectively. Deltapine-14 carried an abundance of mature fruit while *marie-galante* was strictly vegetative. In the greenhouse all plants of both *marie-galante* and Deltapine-14 produced squares and flowers, but even under short-day conditions, *marie-galante* squared about 41 days later and flowered 53 days later than Deltapine-14.

All plants of the F_1 *marie-galante* × Deltapine-14 produced squares in the field and in the greenhouse but the greenhouse plants were about ten days earlier than the field plants. Although all the F_1 plants produced squares in the field, only 49

[2] Throughout this paper the appearance of the first visible fruit form is referred to as squaring, and the anthesis of the blossom as flowering.

FIGURE 2.—Photographs of representative plants from each population of field grown plants: (1) *G. hirsutum* var. *marie-galante*, (2) *G. hirsutum* (Deltapine-14), (3) F_1, (4) F_2, (5) first backcross to *marie-galante*, (6) first backcross to Deltapine-14.

percent developed to the flowering stage and these produced only one or two flowers per plant. Many of the young squares dried up and fell from the plants or otherwise failed to develop after they were initiated. Photograph 3 in figure 2 shows a field grown F_1 *marie-galante* × Deltapine-14 plant. The F_1 resembled the *marie-galante* parent in general appearance although a few small squares and an occasional flower were produced. Under short-day conditions in the greenhouse, all F_1 plants flowered; on the average they were about 17 days earlier than the F_1's which flowered in the field. In the field under long-day conditions the F_2 population segregated into squaring and nonsquaring, and flowering and nonflowering classes. While 88 percent of the field grown F_2 plants squared, only 53 percent developed to the flowering stage. Thus, many F_2 plants showed the same reaction as the F_1 in that squares were formed but did not develop further. As will be seen from figure 3, most of the F_2 plants which squared did so from 55 to 80 days after planting. A few plants pro-

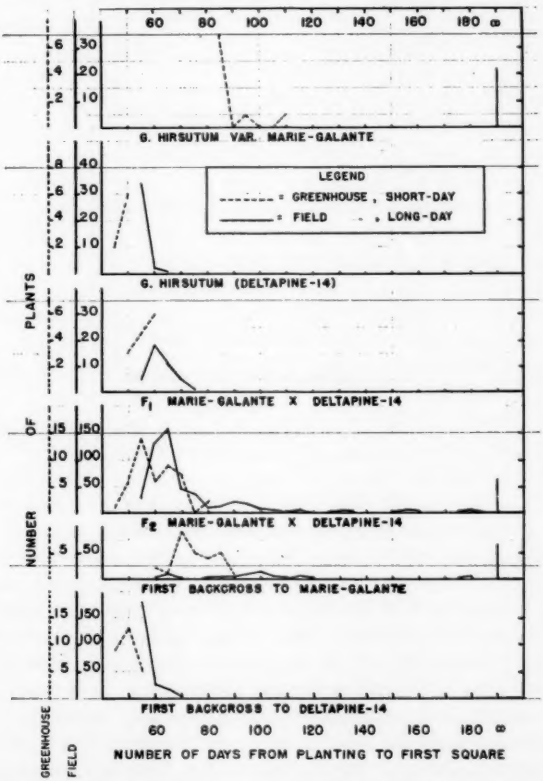

FIGURE 3.—Frequency distributions of number of days from planting to first square for all populations involving *G. hirsutum* var. *marie-galante* and Deltapine-14.

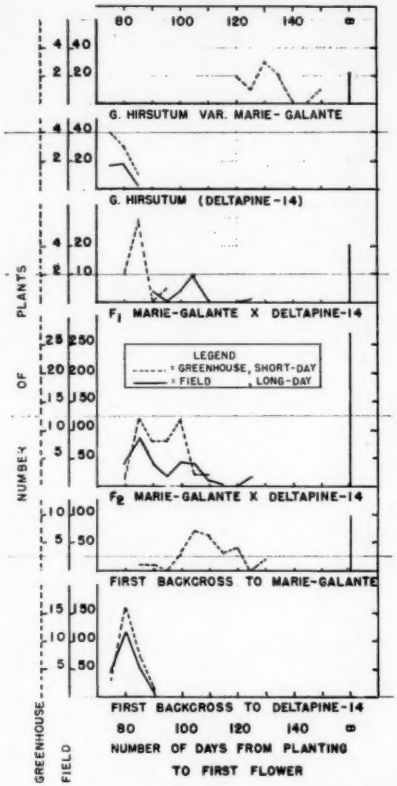

FIGURE 4.—Frequency distributions of number of days from planting to anthesis of first flower for all populations involving *G. hirsutum* var. *marie-galante* and Deltapine-14.

duced squares as late as 180 days after planting and 65 F_2 plants did not square before frost at which time the experiment was terminated.

Figure 4 shows that the F_2 plants flowered from 80 to 130 days after planting, but 298 plants did not reach the flowering stage. Photograph 4 in figure 2 illustrates early and late segregates in the field grown F_2. The early segregate represents a type which flowers early and profusely while the late segregate shows a strictly vegetative condition. The flowering F_2 plants ranged in appearance from the prolific one pictured to the more vegetative types seen in F_1.

The behavior of the F_2 population under greenhouse, short-day conditions was strikingly different from the field planting. All F_2 plants produced both squares and flowers in the greenhouse. Figures 3 and 4 show that all F_2 plants squared by 80 days after planting and flowered by 110 days after planting. There was, however, some segregation for earliness in F_2 in the greenhouse population.

Thirty-five percent of the plants in the field-grown, first backcross to *marie-galante* produced squares, but not a single one produced a flower. The frequency distribution of this population in figure 3 shows that an occasional plant squared but a majority remained strictly vegetative. One such plant is shown in photograph 5 in figure 2; the first backcross to *marie-galante* resembled the *marie-galante* parent. The greenhouse population of the first backcross to *marie-galante* squared and flowered without exception. The squaring and flowering dates of this greenhouse population were intermediate between the F_1 and the recurrent parent, *marie-galante*.

All the plants in the first backcross to Deltapine-14 produced squares and flowers in the field and in the greenhouse. Table 1 and figure 4 show that the plants in the first backcross to Deltapine-14 squared practically as early as those of the Deltapine-14 parent. Similarly, table 1 and figure 4 show that flowering in the backcross to Deltapine-14 was only 3–4 days later than Deltapine-14. Photograph 6 in figure 2 illustrates a typical first backcross to Deltapine-14 with an abundance of mature fruit and numerous fruiting branches.

DISCUSSION

The results of this study showed that *G. hirsutum* var. *marie-galante* and Deltapine-14 differed in their response to the total environment. Deltapine-14 was practically insensitive to the differing environments of the field and greenhouse and squared and flowered in about the same length of time under both conditions. The strain of *marie-galante* used in this study was strikingly sensitive to the same set of environmental conditions; it remained strictly vegetative in the field during the long days of summer and produced fruit forms only in the short days of winter. Due to the lack of facilities for the precise control of light, temperature, humidity and other factors of plant growth, the separate influence of each environmental factor could not be determined. However, in view of the experimental evidence presented here and LANG's (unpublished) work on controlled lengths of day and graft hybrids, photoperiod seems to be the dominant environmental factor in the fruiting response of *marie-galante*. Although the critical photoperiod was not determined it can be said with reasonable accuracy that *marie-galante* flowers only in photoperiods of about ten hours or less and fails to flower in photoperiods of 12 hours or more.

Even after the proper photoperiod for flowering had been provided, *marie-galante* is still inherently later than Deltapine-14 in the production of fruit forms. That this lateness factor is not associated with length of day was revealed by the fact that all greenhouse plants, involving parents, F_1, F_2, and both backcrosses, produced fruit; yet the average time required for squaring and flowering differed significantly among these six populations. It should be pointed out that the winter greenhouse plants grew under lower mean temperatures and lower total radiant energy than did the field plants, and the greenhouse plants never suffered for lack of moisture as did the field-grown plants late in the summer.

All the field-grown F_1 *marie-galante* $\times$ Deltapine-14 plants initiated fruit forms, but only about half of them developed an occasional flower. Assuming that flowering was controlled by the production of some unidentified substance in the plant, the

behavior of the F_1 suggested that the substance was at, or very near, the threshold quantity required for flowering.

A study of the ratios in the field grown F_2 of squaring and flowering plants to those which remained vegetative did not reveal a simple genetic explanation; neither was a typical quantitative type of inheritance exhibited. The data on squaring in table 1 suggests that the difference in photoperiodic response between Deltapine-14 and *marie-galante* is conditioned by one major gene pair with nonflowering recessive. The data deviate from this simplest genetic explanation in that more than three fourths of the F_2 squared while less than one half of the backcross to *marie-galante* squared. It seems likely that a complex of genes which control photoperiod response, in addition to other factors affecting flowering, was operating. The nature of the segregation of the genes in this complex was no doubt masked by the constantly changing length of day. Also, if a threshold value of some substance, conditioned by a complex of genes, must be reached before flowering can occur, a simple classification according to flowering and nonflowering types would not reveal continuous variation below and above this threshold value.

The striking behavior of the two field-grown backcrosses adds further weight to the theory that a threshold level of some flower promoting substance must be reached before flowering can occur. In the backcross to *marie-galante* only 35 percent of the plants initiated fruit forms and none developed to the point of anthesis of the flower. Contrasted with this was the fact that in all backcrosses to Deltapine-14, plants flowered profusely and practically as soon as Deltapine-14 itself. In each backcross the influence of the recurrent parent was dominant. This was to be expected since the F_1 appeared to be very near the critical level. ROGERS (1950) reported a similar tendency for the recurrent parent to be dominant in maize and teosinte backcrosses. This type of inheritance was called "antithetical dominance" by ANDERSON and ERICKSON (1941) where modifiers apparently favor the expression of the recurrent parent's characteristics. It seems likely, then, that *marie-galante* has built up through natural selection a genetic constitution in which its fruiting habit is timed to the short days in the subtropics as suggested by HUTCHINSON, SILOW and STEPHENS (1947). Deltapine-14 is practically insensitive to changes in the environment as regards length of day.

Failure to flower in the normal growing season in the Cotton Belt does not, *per se*, rule out the possibility of utilizing the many cotton introductions having this characteristic. Crosses with nonsensitive strains can be made in the greenhouse during the winter or in a tropical location, and F_2 and backcross seeds can be produced under similar conditions or on a portion of the population when grown under long-day conditions. Early flowering types can be selected in the F_2, and the nonflowering response can be eliminated in the first backcross to a nonsensitive strain. Backcrossing to the sensitive parent would not be successful in a practical plant breeding program, but in all probability character transfer could be effected through backcrossing to a nonsensitive parent.

SUMMARY

The inheritance of flowering response in cotton was studied in a cross between a short-day *Gossypium hirsutum* var. *marie-galante* and Deltapine-14, a day-neutral

strain of *Gossypium hirsutum*. *Marie-galante* remained vegetative when grown in the field during the long days of summer, but set fruit when grown during the short days of winter in the greenhouse. Deltapine-14 set fruit in about the same length of time in both environments.

Under long-day conditions all F_1 plants initiated fruit forms, but only about one half of the plants developed a flower. This showed that neither parent was completely dominant and that the F_1 was very near a threshold level for ability to flower. In each backcross the recurrent parent tended to be dominant, which suggested that the genetic background of each strain strongly influenced the expression of the genes controlling time of flowering. The segregating populations did not reveal a simple genetic ratio; neither was the segregation typical of quantitative inheritance.

Under short-day conditions all plants flowered, but even with a favorable photoperiod *marie-galante* carried a lateness factor not associated with response to length of day.

Failure to flower in the normal growing season in the Cotton Belt does not, *per se*, rule out the possibility of utilizing photoperiodic cottons in genetic and breeding research.

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ON SEX DETERMINATION IN THE DIASPINE SCALE PSEUDAULACASPIS PENTAGONA (TARG.) (COCCOÏDEA)

SPENCER W. BROWN[1] AND FREDERICK D. BENNETT[2]

Department of Botany and Commonwealth Institute of Biological Control, Imperial College of Tropical Agriculture, St. Augustine, Trinidad, B. W. I.

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OF the three sections into which the coccids may be subdivided, the diaspidoid or true scales represent "the largest in point of included species and morphologically the most highly specialized of the entire superfamily" (HUGHES-SCHRADER 1948). Although economically of very great importance, the true scales have been little investigated in regard to either cytology or genetic systems.

SCHRADER (1929) reported a chromosome number of eight for females of *Aspidiotus hederae*, a species which exhibits an obligate type of diploid thelyotoky to be found also in other sections of coccids (HUGHES-SCHRADER 1948). The same chromosome number was reported by DICKSON (1932) for *Aonidiella aurantii* from SCHRADER's counts in embryos of undetermined sex. Resistance of *A. aurantii* to cyanide was found to be inherited; of special significance was DICKSON's demonstration that the males transmitted only the factor or factors received from their mothers. DICKSON interpreted his data as revealing sex-linkage in an XX—XY—scheme; they would obviously conform equally well to a haplo-diploid sex differential.

More recently LINDNER (1954) has reported haplo-diploidy in the diaspidoid scale, *Aspidiotus perniciosus*; the males have four, the females, eight chromosomes. LINDNER also briefly described a typical meiotic sequence in the females and the substitution of a simple mitotic division for meiosis in the males.

The subject of the present report, *Pseudaulacaspis pentagona* was chosen for study because of puzzling facts in regard to its life cycle observed by MR. E. C. G. BEDFORD and BENNETT at the laboratory of the Bermuda Department of Agriculture. These observations have been reported in part in a technical bulletin on mass rearing of scales (DUSTAN 1953) and a further descriptive treatment is in preparation by BENNETT.

Females of *P. pentagona* will lay no eggs unless mated. Following normal matings, their egg production shows a noteworthy example of combined sexual dimorphism and sexual dichronism, or production of the two sexes in two different time intervals. Development of the embryo has advanced considerably by the time the eggs are extruded. Those laid first are coral in color and, as shown by isolation tests, contain female embryos. Without interruption, a second series of eggs is produced; these are pinkish-white and contain male embryos. The two series total 150–200 eggs per female and the sex ratio, as determined in Bermuda, is approximately 1:1. The emergent crawlers also show the same sexual color difference. The work summarized

[1] Fellow of the John Simon Guggenheim Memorial Foundation, and on leave from the Department of Genetics, University of California, Berkeley, Calif. 1956–57.

[2] Present address: Department of Entomology, University of California, Berkeley, California.

in the present report was undertaken primarily to find out the sex determining mechanism in this species.

MATERIALS AND METHODS

Irish potatoes infested with *P. pentagona* were obtained from mass cultures maintained at the laboratory of the Commonwealth Institute of Biological Control located at the Imperial College of Tropical Agriculture, St. Augustine, Trinidad, B. W. I. Culture methods were essentially those outlined by DUSTAN (1953). Original stocks for the mass cultures were taken from *Stachytarpheta cayennensis* in Trinidad.

Virgin females were easily obtained by isolating in screened glass jars infested potatoes from which all male scales had been removed. Production of a secondary covering by unmated females provides a certain criterion of their virginity. Matings were controlled by placing potato pieces bearing pre-emergent males in the isolation jars with the females.

Testes were removed in water and fixed, stained, and squashed in aceto-carmine. For squashes of eggs and embryos, ovarian contents were fixed for one to two hours in Carnoy's fixative consisting of four parts chloroform, three parts absolute alcohol and one part glacial acetic acid. Material for sectioning was fixed in San Felice, embedded in paraffin, cut at 8–12 microns, and stained in crystal violet. When desired, the degree of flattening of the squashes could be controlled by squashing under the dissecting microscope. As a rule, only moderate pressures were used for squashing and, as shown by comparison with the sections, the squashes did not suffer troublesome distortion.

Clinical facilities available for a brief period at a local hospital were used for the X-ray treatments. Dosage was controlled by varying duration of exposure and is believed to have been sufficiently accurate for the comparative purposes of the present report. Males were exposed on thin potato pieces. To prevent death of females through subsequent desiccation of the potato, they were irradiated on uncut potatoes; an approximately flat surface was chosen for exposure and outlined with crayon.

Original negatives of 900× magnification were produced on contrast process orthochromatic film by use of a Leitz Makam camera and enlarged on printing to 2300× (except for fig. 24 which is 1650×). The drawings are diagrammatic sketches made directly from the binocular research microscope (10 × 90 optics) and usually combine details from several focal planes. All illustrations were made from the squash preparations.

OBSERVATIONS

Life cycle of P. pentagona

The pertinent facts in regard to oviposition have already been cited in the introduction. Further information, chiefly in regard to chronology, will be briefly noted here. The duration of each stage is that determined from the mass cultures at St. Augustine, Trinidad.

The eggs hatch about three days after they are laid and the crawlers move out from under the maternal protective covering. The female crawlers scatter themselves widely over the potato surfaces while the males group themselves in tight colonies. Both sexes are ready for mating about 20 days after hatching. The adult, winged males appear in the late afternoon and die by the following morning.

Egg production begins 16–17 days after mating and continues without interruption for about six days. As previously stated, those eggs containing female embryos are laid first. Females live for only a short time after cessation of laying. If unmated, the females begin to build a secondary covering at 3–4 days past the normal mating time. Production of this additional cover will continue for about 15–20 days unless the females mate in the meantime. On mating, production of the secondary covering ceases except for completion of a small section for later sheltering of the eggs.

Cytological observations

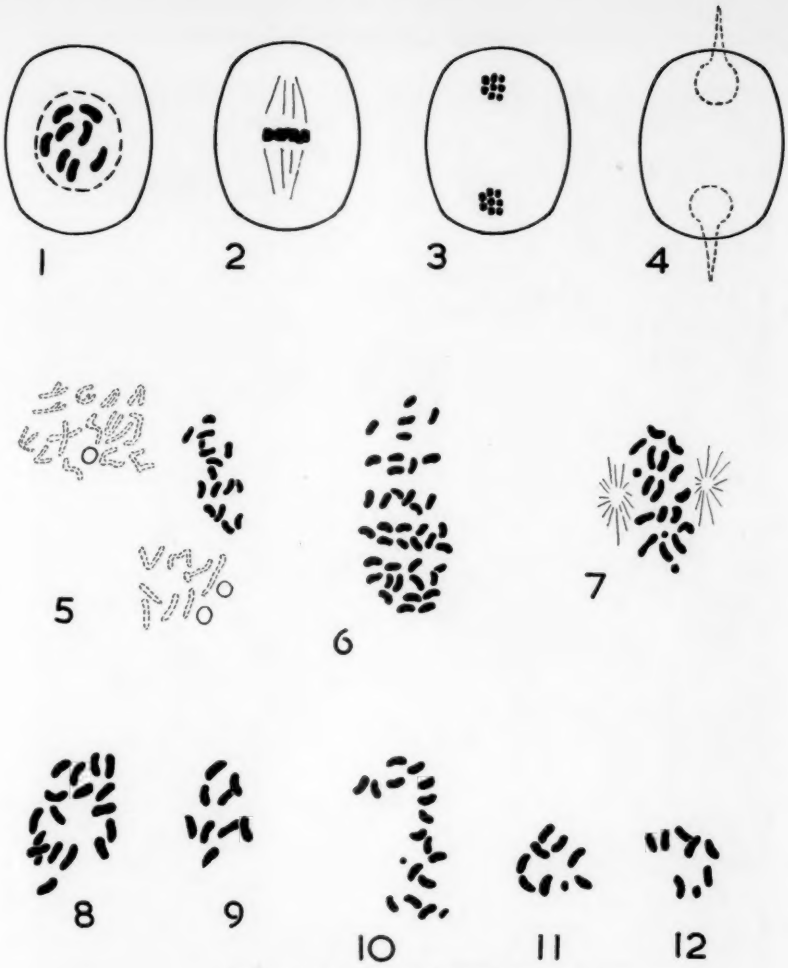
Spermatogenesis. Details of spermatogenesis were obtained solely from squashes. In order to assure observation of all stages, slides were prepared daily over a period of a week from cultures in which age of males varied by only one or two days. The spermatogenic mitosis occurred at about 12 days after hatching or eight days before the males emerged as adults.

A typical resting nucleus precedes the quite clear prophase which marks the onset of the spermatogenic mitosis; undoubtedly only this one mitosis replaces meiosis. The haploid complement of eight chromosomes is easily observed at late prophase (figs. 1, 13) and occasionally in the less highly condensed metaphase plates (figs. 2, 14). The spermatocytes do not divide and sperm formation begins shortly after telophase (figs. 3, 4). The spermatocytes clump together soon after the sperm tails have begun to protrude.

Oogenesis. The ovarioles, each with a single egg, occur along the lateral oviducts and each terminates in three massive nurse cells. The symbionts, yeast-like in appearance, form a collar between the egg and the nurse cells; when the latter undergo dissolution, the symbionts enter the egg protoplasm where they remain in a group at the distal end during the early embryonic stages.

Earlier stages of meiosis were not studied in detail. At late diakinesis, several nucleoli are present and the eight bivalents are quite contracted (fig. 15). The bivalents show an obvious bipartite structure; closer inspection demonstrates that each of the two parts consists of two chromatids. Both products of the first division prepare for a second, but the changes in the first polar body are retarded and it never completes a second mitosis (fig. 16). At the time of fertilization, the egg nucleus is near the center of the cell while the two polar bodies remain a short distance apart from each other, near the surface of the cell and near the distal end.

Origin of polyploid cells. The polyploid cells which appear as a sector of the very young embryo are destined, according to SCHRADER (1923) and WALCZUCH (1932) to harbor the yeast-like symbionts. The later history of the polyploid cells was not followed in *P. pentagona*, but their origin differs somewhat in detail from that previously reported. HUGHES-SCHRADER (1948) has summarized the earlier observations: the two polar bodies unite to form a triploid polar nucleus; division products of the



FIGURES 1–4.—Diagram of spermatogenesis.

FIGURE 1.—Late prophase.

FIGURE 2.—Metaphase.

FIGURE 3.—Telophase.

FIGURE 4.—Early stage in sperm formation.

FIGURES 5–12.—Diagrammatic sketches.

FIGURE 5.—Union of cleavage nucleus (center) with first (upper) and second (lower) polar bodies; chromosomes of polar bodies show varying degrees of contraction and splitting.

FIGURE 6.—Late prophase in cell of polyploid sector of embryo; pentaploid, with 40 chromosomes.

FIGURE 7.—First division of zygotic nucleus with 16 chromosomes and three fragments; from an untreated culture.

FIGURE 8.—Division figure from embryo with 17-chromosomes.

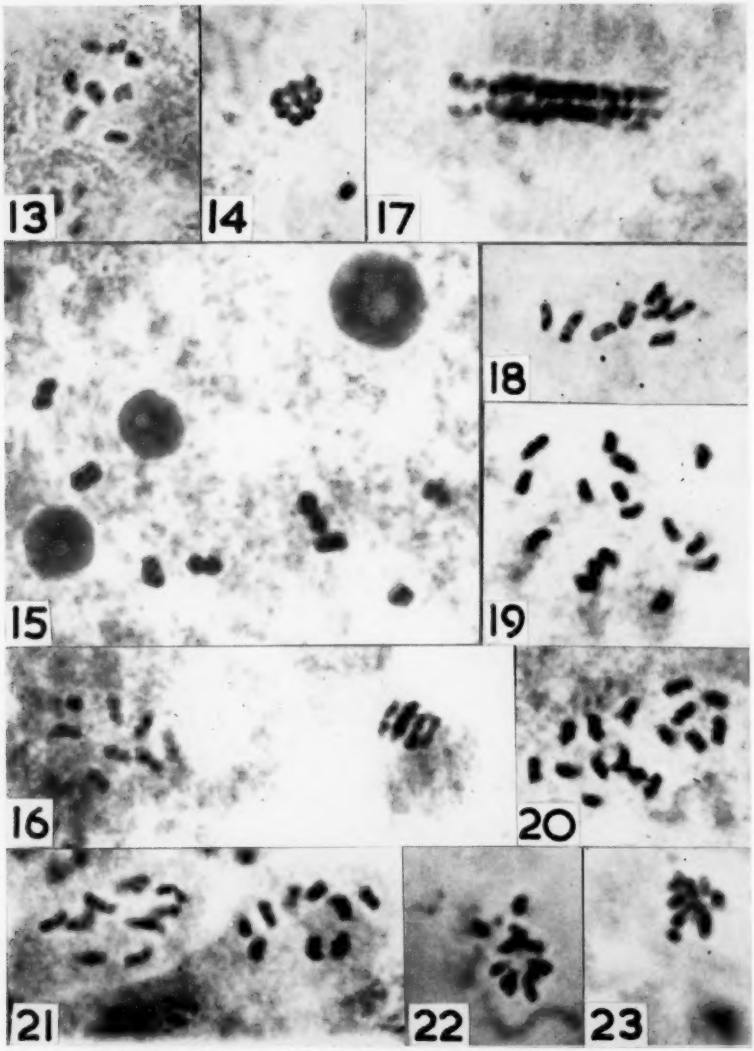
FIGURE 9.—Division figure from embryo with 9-chromosomes.

FIGURES 10–12.—Fragment chromosomes in embryos after parental irradiation.

FIGURE 10.—Diploid embryo with 16 chromosomes and two fragments; father irradiated.

FIGURE 11.—Haploid embryo with eight chromosomes and one fragment; mother irradiated.

FIGURE 12.—Haploid embryo with seven chromosomes and one fragment; mother irradiated.



FIGURES 13–23.—Photographs. See text for enlargements.

FIGURE 13.—Spermatogenesis: Late prophase, eight chromosomes.

FIGURE 14.—Spermatogenesis: Metaphase, eight chromosomes.

FIGURE 15.—Oogenesis: Diakinesis, eight bivalents and three nucleoli (bivalent at lower right oriented at right angles to plane of focus).

FIGURE 16.—Oogenesis: Second division; first polar body (right) does not complete this division.

polar nucleus either unite in pairs to yield hexaploid nuclei or combine with cleavage nuclei to yield pentaploid nuclei.

In *P. pentagona*, the polar bodies remain quiescent until they combine with a cleavage nucleus. Union usually occurs after the fourth or fifth cleavage mitosis, but one example was observed in which a product of the first cleavage mitosis was associated with the polar bodies. After a cleavage nucleus has occupied the space between the two polar bodies (fig. 5), the three nuclei form a single metaphase group in which the contributions of the three components may sometimes be discerned (fig. 17). Chromosome counts from clear, late prophases in the polyploid sector always yielded the pentaploid number of 40 (fig. 6). The triploid and hexaploid numbers of 24 and 48 have not been found; thus the polar bodies in this species do not, as a rule, undergo union and division prior to combination with a cleavage nucleus.

Chromosome eliminations. All embryos in the very early stages of embryogeny have a diploid number of chromosomes. After the establishment of the polyploid sector, chromosome eliminations begin at the proximal end of those embryos destined to become haploid and continue until all or nearly all of the diploid sector has been converted to the haploid state.

The process of elimination is not frequently observed because it is completed during a small part of the mitotic cycle. The chromosomes to be eliminated are not differentiated from the remainder of the set at metaphase or earlier and their condensed mass is ejected from the cell at late anaphase or early telophase. The chromosomes to be eliminated may show some evidence of division and orientation on the spindle (fig. 26), but usually clump into one or two chromatic blobs which are ejected laterally from the spindle (figs. 24, 25). The blobs soon become free from the cells which produce them and are to be observed scattered about the somewhat older haploid embryos. An embryo may show one to several cells in the process of elimination.

Inspection of 8-chromosome embryos at the multicellular stage just prior to laying occasionally revealed a 16-chromosome division figure. It is not known whether such cells stem from escapes from the elimination process or represent a secondarily achieved diploidy. Among the numerous testes examined, however, no diploid spermatocytes were observed.

Chromosomes in embryos. Cytological examinations were made of embryos at various stages prior to laying, and in those in eggs already laid when the sex of the

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FIGURE 17.—Embryos: Early anaphase of the first division of the polyploid nucleus; less contracted chromosomes at the sides are believed to represent contributions of polar bodies.

FIGURE 18.—Division figure of eight chromosomes from a haploid (male) embryo.

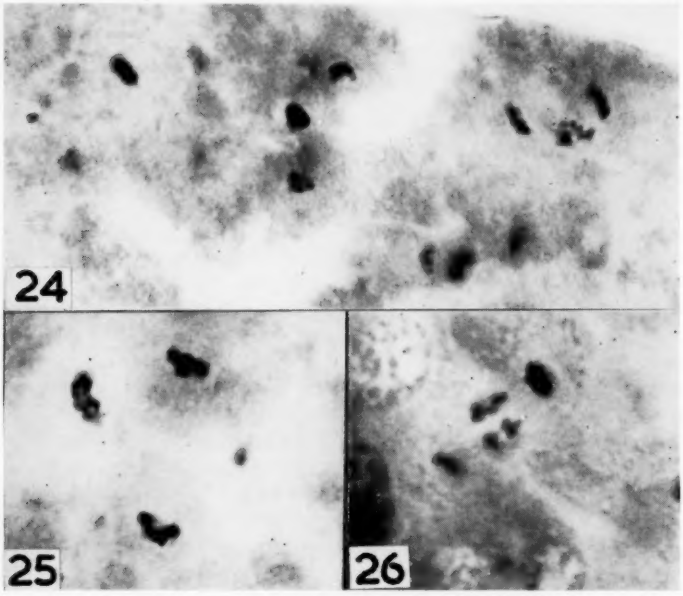
FIGURES 19–20.—Division figures of 16 chromosomes from diploid (female) embryos.

FIGURE 21.—Two division figures of nine chromosomes from embryo with 17- and 9-chromosome nuclei.

FIGURES 22–23.—Chromosome fragments in embryo with sticky chromosomes.

FIGURE 22.—Fragment at 12 o'clock.

FIGURE 23.—Fragment at 7 o'clock.



FIGURES 24–26.—Photographs. See text for enlargements.

FIGURE 24.—Embryo; four division figures showing chromosome elimination.

FIGURE 25.—Embryo; eliminated chromosomes at two sides of spindle; material on right probably represents a single chromosome.

FIGURE 26.—Embryo; eliminated chromosomes in two groups and showing some activity on spindle.

embryo could be determined. In the latter case, the male embryos in the pinkish-white eggs possessed a complement of eight chromosomes; the female, in the coral-colored eggs, one of 16 chromosomes. Examples of the haploid and diploid sets are shown in figures 18–20.

Embryos with an extra chromosome. In several embryos in two females from the same culture, a chromosome number of nine was counted. In two embryos this number was found in several cells in various parts of the embryo and 17-chromosome cells were also present (figs. 8, 9, 21). These observations testify to the regularity of the elimination process. The two females in question had been aged as virgins; since they showed eight bivalents and no univalents at diakinesis, it may be suggested that the aging process sometimes disturbs the regularity of segregation.

Spontaneous chromosome fragmentation. Broken chromosomes were found in three of the hundreds of embryos obtained from untreated cultures. Two are of interest. In the first, the fragments appeared in the prophase of the first division (fig. 7). In the second, at a late cleavage stage, the division figures appeared "sticky" and showed occasional fragments (figs. 22, 23).

An X-ray experiment

Because of the diffuse kinetochore, X-ray induced fragments of coccid chromosomes can perpetuate themselves through many cell generations (HUGHES-SCHRADER and RIS 1941). Broken chromosomes should therefore be utilizable as genetic markers to reveal the origin of the haploid chromosome set of the male embryos. On assumption of maternal derivation of the haploid set, the chromosomes of the male embryos should be fragmented only after maternal treatment and the number of males should be reduced by induction of lethal changes. Conversely, similar results would be expected following treatment of fathers on assumption of paternal derivation. The results obtained in regard to chromosome fragmentation conformed to expectation but, in regard to lethality, they were complicated by a quite unexpected effect on sex ratio.

Origin of the male chromosome set. Cytological observations of 109 embryos in the ovaries of eight females are summarized in table 1. Four of these females were irradiated and then mated to untreated males (females 1–4); the other four were not treated themselves but were mated to treated males (females 5–8). The irradiations were made 2–3 days prior to mating and the embryos were examined a few days prior to the time for their extrusion.

After irradiation of the mothers (table 1, females 1–4) fragments (figs. 11, 12) were found in 14 of the 42 8-chromosome embryos examined, or in one third of the instances. Only a few 16-chromosome embryos, presumably females of retarded development, were present in the ovaries at the time of fixation; one of these, from female 2, showed fragmentation. After irradiation of the fathers, fragments were found in only the 16-chromosome embryos (fig. 10); no fragments were found among 43 8-chromosome embryos. A statistical comparison may be made of the 14:28 ratio for maternal radiation with that of 0:43 for the paternal: χ^2, with Yates' cor-

TABLE 1

Chromosome fragments in embryos from eight females after X-ray treatment of one of the parents

Parent treated and dose	Embryos from female no.	Eggs laid prior to fixation[1]		Fragments in male embryos[1]		Fragments in female embryos[1]	
		F	M	Absent	Present[2]	Absent	Present[2]
Mother, 3000r	1	0	23	3	5	(none)	
	2	26	25	10	2	1	1
	3	4	1	4	3	1	—
	4	(not det'd)		11	4	(not det'd)	
Father, 5000r	5	ca. 20	0	(not det'd)		6	4
	6	ca. 20	0	23	0	1	6
	7	0	20	11	0	(none)	
	8	ca. 20	0	9	0	1	3

[1] Sex determination was based on color of the embryos in the eggs produced prior to fixation and on chromosome number in the embryos of unlaid eggs. All cytological examinations were of embryos in unlaid eggs, from a few hours to several days prior to laying.

[2] The number of fragments ranged from one to two per cell in the male embryos, from one to five per cell in the female. The most frequent number in both was one.

TABLE 2

Sex of embryos at oviposition and percent hatching following X-ray treatments and aging

Line no.	Treatment[1]		No. females examined	Av. no. embryos[2]			Percent hatched
	Maternal	Paternal		F	M	Total	
1	0r	0r	9	83.4	48.1	131.5	90.5
2	0r	5000r	20	32.7	101.9	134.6	84.3
3	1285r	0r	5	18.0	119.2	137.2	91.8
4	3000r	0r	13	13.2	53.8	67.0	84.6
5	Aged[3]	—	12	1.6	82.7	84.3	—

[1] The X-ray treatments (lines 1–4) were all given at the same time; the experiment on aging (line 5) was conducted at a later date.

[2] Sex determination was based on color of the embryos in the eggs as they were laid.

[3] Females were not mated until 20 days after sexual maturity.

rection, is 14.8; df = 1, and $p < 0.01$. The high frequency of chromosome breakage in the females following paternal irradiation testifies to the effectiveness of this treatment; the statistical comparison is therefore believed to be valid for the present comparative purpose. The conclusion to be drawn from this analysis is that it is the paternal chromosome set which is eliminated during development of male embryos.

Effect on sex ratio. The number and hatchability of eggs produced after irradiation of one of the parents are listed in table 2. Counts were made daily. It was necessary to raise the protective covering of the mothers, and this disturbance may have been responsible for shortening the laying period and thereby affording a preponderance of females in the control cultures (table 2, line 1).

Irradiation of either parent results in a marked decrease in the number of females. Such a result may be expected as a consequence of paternal irradiation followed by the transmission of dominant lethals to the daughters and nothing to the haploid set of the sons (with the exception, of course, of dominant lethals active during the early, diploid phase of the male embryos). Although the results obtained by paternal treatment conform to this expectation (table 2, line 2) the unexpected results of maternal irradiation indicate that caution in interpretation is required.

When mothers are irradiated, the sons would be expected to be preferentially affected because the haploid set will manifest recessive as well as dominant lethals. The expected result was quite clearly not obtained (table 2, lines 3 and 4). It seems likely that the effect on sex ratio may be attributable in large part to factors other than genetic.

The absolute increase in number of males noted in two cases (table 2, lines 2 and 3) is probably attributable simply to the fact that the female will lay about the same total number of eggs regardless of their sex unless a very large number of embryos is damaged. It should be noted that none of the totals is as large as twice the number of females produced by the untreated parents (table 2, line 1) or a calculated total of 166.8. The decrease in total number following the heaviest maternal treatment (table 2, line 4) may be in part attributable to damage to eggs developing at the time of irradiation; at sexual maturity of the mother most eggs have yet to complete their meiotic divisions.

As measured chronologically, the embryos of this species have completed about 80 percent of their prehatching development at the time of laying. The hatchability of the eggs is little affected by the X-ray treatments (table 2). It seems likely that only those eggs are laid in which the embryos have succeeded in reaching a required stage of development. The decrease in hatchability found with the higher doses of X-rays (table 2, lines 2, 4) may consequently be the result of lethal factors expressed only in late embryonic development.

Sexual dichronism and effect of maternal aging

During earlier studies in Bermuda, BENNETT amassed a large series of data from isolation tests on the sexual dimorphism and dichronism of *P. pentagona*. These data, which were unfortunately lost, have already been summarized in the introduction of this report. The sexual dichronism is so strictly regulated that it has been put to practical purpose in the efficient rearing of large numbers of scales. From a tray of potatoes carrying uniformly aged females it is possible to infest other trays of potatoes almost exclusively with either female or male crawlers.

In the present series of experiments only a very few eggs were produced out of phase. Several of these appeared following treatment in the X-ray series as single female eggs produced after two or three male eggs had been laid. In the ovarian cytological materials, the 16-chromosome embryos were invariably more advanced developmentally than those with eight chromosomes except for a few instances in the X-ray series.

When females are prevented from mating for a considerable time after sexual maturity, the large majority of their progeny are male (table 2, line 5). Inspection of ovarian contents of aged females during the days following mating showed no abortion or degeneration of embryos and the most advanced embryos, in the limited series counted, all had eight chromosomes. The change in sex ratio cannot, therefore, stem from differential mortality. It seems likely that it results from a much earlier onset of chromosome elimination in the series of developing embryos.

In the aging experiment summarized in table 2, line 5, the 12 females produced a total of 990 eggs of which only 22 were female. One mother laid 15 of the 22; the residual percentage of female eggs is less than one and this small percentage could easily have escaped detection in the cytological examination just described. Of the 22 female eggs, five were produced out of phase by two mothers. The mother which produced 15 female eggs gave the following sequence: 13 female, 53 male, and 2 female; from the second mother, 3 female eggs followed 43 male eggs. The few late appearing female eggs seem most readily explained as escapes from the conversion of normally diploid to haploid embryos; their occurrence thus helps to support the conclusion that aging affects sex ratio by predisposing most of the eggs to later chromosome eliminations.

Cytological conditions in other genera

FERRIS (1937–1942) states that, "On the whole the Diaspididae are quite a homogeneous group, . . ." In addition to the three reports available from the literature, and the subject of the present report, six more determinations have been made from scales which were, at least for the gravid females, readily available at St. Augus-

TABLE 3

Chromosome numbers of some diaspine scales

Species, and lit. cit., unless new	Tribe[1]	Chromosome numbers of				
		Female	Male	Embryos		
				Higher	Lower	Polyploid sector
Aonidiella aurantii (Mask.) (Dickson, 1941)	A	—	—	(8)[2]	—	—
Aspidiotus perniciosus Comst. (Lindner, 1954)[3]	A	8	4	—	—	—
Aspidiotus hederae (Vall.) (Schrader, 1929)	A	8[4]	—	—	—	—
Aspidiotus destructor Sign.	A	8	4	8	4	20
Aspidiella hartii (Ckll.)	A	8	—	8	4	20
Chrysomphalus ficus Ash.	A	8	4	8	4	20
Selenaspidus articulatus (Morgan)	A	8	—	8	4	—
Pseudaulacaspis pentagona (Targ.)	D	16	8	16	8	40
Lepidosaphes beckii (Newman)	D	8	4	8	4	20
Diaspis bromeliae (Kern.)	D	8	4	8	4	—

[1] A—Aspidiotini; D—Diaspidini.

[2] From embryos of undetermined sex.

[3] Synonym of *Quadraspidiotus perniciosus* (COMSTOCK) (FERRIS 1937–1942).

[4] Parthenogenetic females only.

tine. These chromosome number determinations are summarized in table 3. The basic number, n = 4, seems to be characteristic of the two main tribes of the diaspine subfamily, the Aspidiotini, and the Diaspidini. *Pseudaulacaspis pentagona* is obviously a tetraploid species and remains the only exception so far found to the numerical uniformity. SMITH (1941, 1942) reports the occurrence of a tetraploid species in the Diprion sawflies, hymenopterous forms with a haplo-diploid sex differential, and his findings are further discussed by WHITE (1945).

In two of the genera which were cursorily surveyed, *Selenaspidus articulatus* and *Chrysomphalus ficus*, chromosome elimination seemed to follow a somewhat different sequence from that described above for *P. pentagona* in that the set to be eliminated appeared to be heteropycnotic during late prophase.

In conclusion, it seems likely that a haplo-diploid sex differential is characteristic of the two main tribes of scales and that this differential is probably achieved through chromosome elimination. The extent to which the elimination is controlled by aging or maturing processes in the ovaries and expressed as sexual dichronism in scales other than *P. pentagona* is not yet known, although the otherwise uniform cytological picture would indicate that this mechanism of sexual predetermination may prove to be characteristic of the diaspine scales.

DISCUSSION

The results described above for *P. pentagona* may be summarized as follows in regard to sex determination. Fertilization is required for the production of both male and female embryos. Females are diploid; males, haploid; the haploidy of the males is achieved through elimination of the paternal chromosome set during late

cleavage divisions. The embryos destined to undergo chromosome elimination form the latter group produced by the mothers. Since aging the mothers prior to mating markedly alters the sex ratio in favor of the males, it may be suggested that physiological processes similar to those produced by aging occur normally and rapidly in ovaries once embryos have begun to develop. The maturing or aging processes may be looked upon as inducing or controlling chromosome elimination and therefore as factors predetermining sex while sexual determination itself is brought about by the haplo-diploid differential.

The unique aspect of the scheme just described is the establishment of a haplo-diploid sex differential through a mechanism of chromosome elimination. The various parts of this system are familiar if not from the coccids themselves, at any rate from other insects.

Haploidy of males has been described in detail for several genera of iceryine coccids (HUGHES-SCHRADER 1948) and spermatogenesis in *P. pentagona* conforms to that described for the other haploid coccids.

Chromosome elimination during cleavage stages is familiar from Sciara (METZ 1938). In this dipteran, the large "limited" chromosomes are excluded from the soma. Of special significance for the present case is the predetermination, by the genotype of the mother, of the elimination of sex chromosomes during cleavage. Three X chromosomes are present in the zygote; the predetermined differential eliminations result in an XX-soma for females and an X-soma for males. Of closer relationship than the diptera are other homoptera, certain aphids and phylloxerans (MORGAN 1915) in which the smaller of two size classes of eggs (dimegaly) is predestined to produce male offspring; sex chromosome elimination occurs during the meiosis which precedes the development of parthenogenetic males.

Preferential elimination of paternal chromosomes has been shown to occur in Sciara (METZ 1938) both during cleavage divisions, when sex chromosome elimination occurs, and in spermatogenesis when the remaining paternal chromosomes are discarded.

Maternal aging as a factor in chromosome elimination is apparently not known from nature but has been shown to be of significance in increasing the elimination of a ring-shaped X chromosome of *Drosophila melanogaster* (BROWN and HANNAH 1952). More recently HANNAH (1955) has shown that similar aging will also bring about a very marked increase in elimination of the normal, rod-shaped X chromosome although the regularly observed instability of the rod X is very much less than that of the ring X.

Bearing on lecanoid sex determination. The results of the present study are of special interest in connection with the cytological observations reported for the specialized lecanoid coccids (HUGHES-SCHRADER 1948). In the several species studied, the two sexes have the same diploid chromosome number. In the males, however, one set becomes heteropycnotic in the late blastula stage and is eliminated during meiosis; it has been suggested that the heteropycnosis of one chromosome set results in a physiological haploidy of the males.

JAMES (1937, 1938) reported a very striking effect of delayed mating on sex ratio in a lecanoid, *Pseudococcus sp.*; maternal aging gave a great increase in percentage

of males and this increase could not be explained by differential mortality, at least for the shorter aging periods.

On analogy with *P. pentagona*, sex in the lecanoids would be predetermined by physiological factors producing not elimination but heteropycnosis of the paternal chromosome set of the male embryos. Further investigation of the specialized lecanoids in regard to a possible sexual dichronism and to the cytological results of maternal aging would seem to be warranted.

Maternal aging and irradiation. Numerous demonstrations have been presented, especially from plant material, of a similarity of deleterious effect on chromosomes of aging and X-ray treatment; a review of this work is outside the scope of the present report. HANNAH (1955) has recently demonstrated that maternal aging influences the sex ratio of *Drosophila melanogaster* in a manner parallel to that of X-ray treatments whether ring or rod X chromosome stocks are employed.

In the case of *P. pentagona* aging of unmated females has an effect on the subsequent sex ratio similar to that of X-irradiation of the mother (table 2, compare lines 3 and 4 with 5). On a simple genetic basis, the opposite effect of maternal irradiation would be expected; the haploidy of the sons should make them more susceptible than the daughters to the presence of lethal factors. The ovarian contents of the irradiated females showed abortive embryos which could not be classified as to sex; the possibility cannot be eliminated, therefore, that the sex ratio may be modified by a higher mortality of females than males when development occurs in previously irradiated protoplasm. On the other hand, diploids might be presumed to be less sensitive than haploids to a damaged protoplasmic milieu. It thus seems more likely that maternal irradiation predisposes the eggs to subsequent chromosome eliminations rather than to a preferential mortality of the daughters. Promise that this question may be answered conclusively by the results of X-ray treatments at low doses comes from a consideration of the marked effect on sex ratio of the relatively low dose cited in table 2, line 4.

SUMMARY

In the diaspine scale, *Pseudaulacaspis pentagona*, the males are haploid, with eight chromosomes, and the females are diploid, with 16. Meiosis is normal in the female and replaced by a simple mitosis in the male.

Mating is required for the production of any offspring, either male or female. Egg production affords an example of combined sexual dimorphism and dichronism; the mothers first produce a series of eggs containing coral-colored female embryos and then, without interruption, lay a series containing pinkish-white male embryos. Cytological study of early embryogeny shows that a chromosome set is eliminated to produce the haploid condition of the male embryos. Chromosomes broken by X-rays were used as genetic markers to demonstrate that the eliminated set is of paternal origin.

Symbionts, yeast-like in appearance, are present at the distal end of both male and female embryos. The polyploid sector of the embryo stems from the fusion of a cleavage nucleus with both polar bodies and is pentaploid with 40 chromosomes.

As would be expected on a simple genetic basis, irradiation of fathers preferentially reduces the number of daughters. A similar effect on sex ratio following maternal

irradiation cannot be explained on simple genetic assumptions and is believed attributable to a physiological effect of X-rays in predisposing the eggs to chromosome elimination. Aging of mothers prior to mating also results in a marked increase in the proportion of sons; since differential mortality may be ruled out by inspection of ovarian contents, earlier onset of chromosome elimination is believed to be responsible for the effect of aging on sex ratio.

Several other genera of the two main tribes of North American scales, the Aspidiotini and the Diaspidini, have a haplo-diploid sex differential but with the chromosome numbers 4 and 8. The problem of sex determination in the lecanoid coccids, in which one chromosome set is heteropycnotic in the males, is considered in the discussion.

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FITNESS AS A MEASURABLE CHARACTER IN DROSOPHILA

G. R. KNIGHT[1] AND ALAN ROBERTSON[1]

Institute of Animal Genetics, University of Edinburgh

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THE discussion of evolutionary problems in terms of modern genetics involves the concept of the "fitness" of different genotypes—the average ability of organisms to pass on the genes they carry to the next generation. By variations in "fitness", the array of gene frequencies is shaped from generation to generation.

As a measurable character, it is to the quantitative geneticist one extreme of the range, a character of whose function in natural selection there can be no doubt. Despite its theoretical importance, there has been surprisingly little experimental work devoted to direct measurements of fitness. There is notable work in which the relative fitnesses of different genotypes have been inferred from changes in gene frequency (for instance LUDWIN 1951; REED and REED 1948; and the work on Drosophila inversions by DOBZHANSKY and his co-workers) but we are here concerned with the inverse problem—the inference of changes in gene frequencies (and in some cases the stability of gene frequencies) from experimental observations of the fitness of individuals.

There are several important problems in which such measurements are of obvious value. In recent years there has been considerable interest in the effect on populations of an increase in the mutation rate (particularly by irradiation). As the genetic situation is shaped by the relative fitness of the different genotypes, so we can usefully discuss the effect of changes in the mutation rate only on fitness itself. There has been much theoretical speculation on this point which requires experimental work to check it.

As the ultimate character, fitness is of special importance to the quantitative geneticist, and its response to inbreeding, for instance, is of considerable theoretical importance. Again, the interrelationship of fitness with other characters is fundamental to a discussion of the genetic control of such characters in wild populations. LERNER has recently drawn attention to these problems in his book on *Genetic Homeostasis* (1954) in which he discusses the evidence about the mechanism by which such large amounts of genetic variation are maintained in populations.

But what is fitness? For the individual, it is simply the number of offspring in the next generation; for the genotype, it is most aptly described by FISHER'S phrase (1930) the "expectation of offspring". Other terms have also been used such as survival value, selective advantage, reproductive rate, adaptive value and so on. Unfortunately, several of these have misleading connotations which have led to some controversy [see, for instance, the discussion between LI (1955) and CAIN and SHEPPARD (1956)]. "Fitness" in the sense that it will be used here refers to a property of individuals or an average value for individuals measured in a group. In so far as we shall later be discussing such groups or subpopulations, we shall solely be con-

[1] Member of the Agricultural Research Council's scientific staff.

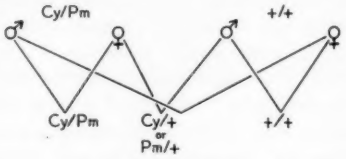
cerned with the individuals making up the group and in no way with the ability of the group to survive as a closed population.

It is operationally convenient to break down the total fitness into the chance of survival to maturity (measured in Drosophila at the point of emergence) and into the fertility after maturity. The latter will refer to the whole mature life of the animal and will therefore include longevity. We may regard these two—early survival and subsequent fertility—as the "components" of fitness. For a male, fertility consists of ability to impregnate females—male mating ability. It may be noted that though male mating ability is undoubtedly an object of natural selection within species, it contributes little to the ability of the species to survive as a unit, provided it is above a certain limit. If a Drosophila female is to lay eggs for three weeks, it presumably affects her output little whether she is impregnated at one hour or at five hours after emergence. As a further consequence, willingness of a female to mate will have little selective advantage within species and her fertility will then be determined mostly by the total number of eggs she lays and their hatchability, in so far as it is a property of the female. These fitness components are biologically distinct entities and must be combined to give total fitness. They are not alternative measures of fitness, as WALLACE (1952, Table 9) seems to imply. Observations on Drosophila populations have only too often concentrated attention on survival to emergence, often in the form of segregation ratios. There is still very little information as to which of the components are most important in controlling the variation in overall fitness and we are here concerned mostly to present a simple method of measurement which includes all components. We shall see later that it combines them in the wrong way but it is satisfactory for a preliminary attack on the problem.

If we are to count the average number of offspring left by individuals, at what stage should the counting be done? It is first of all obvious that the two generations must be counted at the same point in the generation cycle. As we are concerned with differences between genotypes, we should count at the moment the genotype comes into existence—at the moment of fertilization. This raises obvious practical difficulties. But we shall here be concerned with the average fitness of individuals forming stable breeding groups. Any point in the generation cycle will then be logically satisfactory as the separate generations will be genetically identical, and wherever in the generation cycle we start, we shall be including just the same processes but in a different order. It is convenient to count at the point of emergence and this fits in well with our breakdown of total fitness into survival to emergence and subsequent fertility. In dealing with such groups, we can also evade the problems posed by genes like fused (LYNCH 1919) and grandchildless (SPURWAY 1949) whose effects on fitness are not complete when their bearer may be dead.

The technique that we have developed does take into account all the biological components of fitness, though it has some drawbacks which we shall consider later. The basic method is to allow the stock to be tested to compete in equal numbers against a standard tester stock. The latter is chosen so that the 'purebreds' of either stock can be distinguished from the crossbreds. The fitness measurement is then derived from the relative numbers of the two purebred stocks which emerge. The tester stock that we have used is the well-known second chromosome lethal testing

stock, *Cy/Pm*. This contains two easily scored dominant markers balanced against each other by inversions to suppress crossing over and lethals within the inversions to eliminate the homozygotes. The process of competition with a wild type stock can be envisaged as follows



The fitness measurement (for which we shall use the noncommittal term "competitive index") will, if the tester and tested stocks have been put in in equal numbers, simply be the ratio of the numbers of $+/+$ and *Cy/Pm* flies emerging.

The procedure starts with the setting-up of cultures containing a mixture of fertilized *Cy/Pm* and $+/+$ females. The ratio of the two types will depend on the stock we wish to test but with outbred wild stocks we have used a *Cy/Pm*:$+/+$ ratio of 2 to 1. When the progeny emerge, balanced groups of males and virgin females are collected from each emergence. In other words, if we have to set out competitive cultures in a 1 to 1 ratio, both in males and females equal numbers of the two types are taken from each hatch and the remainder discarded. From these groups (equivalent as far as the two types are concerned for age and parental culture) the competitive cultures are started, generally with 50 males and 50 females in a half pint bottle. The parents are discarded after five days and the progeny counted for the first four days of full emergence. In this period, probably about 80 percent of all flies will have emerged. The competitive index of the competing stock is given by the relative numbers of $+/+$ and *Cy/Pm* flies, which obviously depends on the relative viability from egg to adult, male mating ability and female egg laying ability of the two stocks. The ratio of *Cy/Pm* to $+/+$ flies in the competitive cultures can, of course, be varied. With our standard outbred stock, we have found that a 2 to 1 ratio of *Cy/Pm* to $+/+$ in both sexes of the parental flies gives approximately a 1 to 1 ratio in the progeny. The resultant index has of course to be modified according to the ratio of parental types used.

As an example of the use and value of the technique, we can instance some observations on the effect of inbreeding in our standard outbred strain of *D. melanogaster*, the Kaduna strain from Nigeria. This strain has been used in the series of experiments on selection for abdominal bristles (see CLAYTON, MORRIS and ROBERTSON 1957). The competitive index of the Kaduna strain was measured using a 2 to 1 ratio in both sexes. In the series, ten replicate cultures were set up (with different samples of parents) and counts for the first four days of full emergence are given in table 1.

The proportion of wild type flies, expressed in terms of all "pure" flies, was 0.483 with a standard deviation between replicates of 0.136 and a corresponding standard error for the mean of 0.043. The competitive index of Kaduna compared to *Cy/Pm* is thus $4 \times \frac{0.483}{0.517} = 3.74$ with a standard error of 0.64.

TABLE 1

The competitive index of the Kaduna strain

Replicate	1	2	3	4	5	6	7	8	9	10
+/+	97	177	156	71	137	114	61	93	56	97
Cy/Pm	51	70	129	125	118	99	91	134	123	181
+/+ / Total "pure"	0.65	0.72	0.55	0.36	0.54	0.54	0.40	0.41	0.31	0.31

TABLE 2

The competitive index of inbred lines relative to their parent population

Line no.	Competitive index	Line no.	Competitive index
2	0.65	10	0.12
3	0.16	11	0.05
4	0.03	12	0.05
5	0.19	13	0.10
7	<0.01	14	0.01
9	0.22		
		Average	0.14

It may be felt that the accuracy is not high, bearing in mind the number of flies scored. The standard deviation between replicates corresponds to that of a binomial distribution with n = 13. It seems that the effective sampling unit is the first insemination of each individual female (which may not be inseminated more than once in the laying period) rather than the individual progeny scored, as was found by KNIGHT, ROBERTSON and WADDINGTON (1956) in a similar experiment on sexual isolation. It follows that increased accuracy could most easily be obtained by increasing the number of parental females in each culture.

Two series of replicates of similar size but with parental ratio 1:1 have given values for the competitive index of the Kaduna stock of 4.52 ± 0.82 and 3.21 ± 0.52, compared to our standard *Cy/Pm* stock. Bearing in mind the standard errors of the estimates, the agreement is reasonable.

At the time when the method was developed, we chanced to have available a series of inbred lines made from this Kaduna wild type stock. They were the survivors of 100 lines which had been carried on by continued full-sib mating, with only one pair-mating in each line. At the eighth generation of inbreeding, they had been reduced in number to 14 and steps were taken to preserve this remainder, by keeping three pair-cultures in reserve in each line. These were thus the survivors of an intense selection pressure during the process of inbreeding. At the twelfth generation of inbreeding ($F \sim 0.90$), the competitive index of 11 of the lines was measured by the technique already described. As it was suspected that they would be low in fitness, the competition cultures contained equal numbers of inbred and *Cy/Pm* flies. Two replicates were taken in each line.

The results are summarized in table 2, in which the competitive index is expressed as a fraction of that of the parent outbred population.

TABLE 3

Comparison of competitive index and "adaptive value" (based on second chromosome segregation) for three inbred lines (expressed in relation to the noninbred population)

Line	Competitive index	"Adaptive value"
4	0.03	0.83
7	<0.01	0.69
14	0.01	0.76

Differences between replicates were similar to those in table 1 though a statistical analysis is difficult because of the varying ratios in the separate lines. With the exception of line 2, the inbreeding has produced a very marked decline in competitive index, in spite of the selection during the formation of the lines. However, the general relevance of these results is best left to another place, as we are concerned here in the main with the technique itself. It may be noted that, notwithstanding the low competitive index of some of the lines, none was difficult to maintain in mass cultures and the low index came as a surprise to us.

In addition to the measurements on the competitive index, we mated together the *Cy*/+ flies from the competition bottles to measure the segregation ratio of *Cy*/+ and +/+ flies in the lines 4, 7, and 14, which had shown the worst competitive index. This method measures the effect of second chromosomes from the tested stock on survival from fertilization to emergence and has been used by WALLACE and KING (1951) as a measure of the "adaptive value" of their irradiated populations. The "adaptive value" is then based on the ratio of +/+ to *Cy*/+ flies segregating. The comparative results are shown in table 3.

We shall see later that our technique certainly overestimates differences between populations in fitness whereas that used by WALLACE must underestimate them, as it measures only the effect of part of the genetic material on part of the reproductive cycle. Nevertheless the discrepancy between the two measures on these three lines, which were picked out solely because they had a low competitive index, is startling.

We might also mention here some results which perplexed us at the time but which have now come more into focus. Our second test of the outbred population, on the same scale as the first, gave an index relative to the *Cy*/*Pm* tester of only 1.18, compared to the first value of 3.74. It was however then realized that this subpopulation had been kept in bottles for 15 generations, with a mean parental number of about 20 pairs, which would lead to an inbreeding coefficient of around 40 percent. The parent stock, in which the population size is around 5000, was then retested along with the subpopulation in bottles. The former gave a value of 4.52, as reported above, and the subpopulation again gave a value of 1.2. It seems that we can ascribe the lower index of the subpopulation to the inbreeding that it has received.

Shortcomings of the technique: The main purpose in developing this technique was to find a simple measurement involving all three components of fitness—survival to emergence, and male and female fertility. But as a method of measuring fitness itself, the technique has the drawback that the components are put together in the wrong way. The fitness of an animal (or of a group of animals) is their "expectation of

offspring" and can be split into the chance that they will survive to breeding age and into the average number of offspring of those reaching that age. In formal terms $W = SF$ where S is the chance of survival to breeding age and F the fertility of those surviving. F will naturally include longevity. If S is the same for both sexes, then for the average fitness of the population we have $W = \frac{S(F_m + F_f)}{2}$, the subscripts referring to sexes.

Now, in our tests, if we take as unity the separate components measured on the *Cy/Pm* stock, the relative numbers of eggs laid by wild type and *Cy/Pm* females, each fertilized by their own type of male, is $F_m F_f$ and, from these, the relative numbers of the two types emerging is $SF_m F_f$. Thus, in this technique, the components are combined differently from a true fitness measurement and, for this reason, we have used the term "competitive index". Consideration of the two formulae will show that differences between stocks in "competitive index" will on the whole be greater than for true fitness but that the two will in the main have a high correlation. In a sense, differences in fertility between stocks are given too much weight in the "competitive index". Perhaps the most satisfactory solution to the problem would be to measure the three components separately and to combine them in the correct manner. This would, however, be considerably more laborious than the technique presented here.

Two other important defects of the technique are obvious. The first is that, in allowing eggs to be laid over only five days, little account is taken of longevity. This can, of course, be overcome by transfer of the parents to further bottles but transfer greatly increases the time taken. We have not considered the extra labor worth while. The existence of any degree of sexual isolation between the stocks placed together in the competition bottles would wreck the technique but fortunately few cases of such isolation have been observed within species. However, if the technique is to be used in any series of experiments, other tester stocks will have to be investigated.

On a more trivial level, it is obvious that the results obtained will be greatly dependent on the precise external conditions under which the competition is carried out and therefore do not in any way give an absolute figure. The most important of these are the number and age of the females in competition, the period for which they are allowed to lay eggs, the period over which emerging flies are counted and, of course, the nature of the food supplied. The exact conditions used will depend on the particular problems being investigated. In determining the feasibility of the technique, we have been to some extent guided by experimental convenience rather than undue inflexibility. One desirable modification would be to increase the number of females laying in each bottle as this is the main factor causing deviation between replicates.

SUMMARY

A technique for examining the relative fitness of different stocks is put forward. It involves competition with a stock marked with two dominant genes and the final assessment is given the term "competitive index". Examples are given of its use in measuring the effect of inbreeding on an outbred population. Shortcomings and possible modifications of the technique are discussed.

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